

Gen5™ & Gen5 Secure User's Guide

Microplate Data Collection & Analysis Software

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Notices

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About this Guide

This user guide is intended for licensed users of Gen5™ and Gen5™ Secure, BioTek's microplate data collection and analysis software.

Document Conventions

❖ Special or important information uses this format to call your attention to it.

💡 Tips or suggestions, for example, to improve performance, are shown in this manner, following a light bulb graphic.

Navigation instructions for the current topic are presented in this format

Document History

Revision	Date	Changes
A	May 2006	Initial Release
B	Sept 2006	New features related to the Synergy™ 2 reader and Filter Wheel Library
C	March 2006	Added description of new features and enhancements released in Gen5 version 1.02

Chapter 1

Initial Setup

This section provides instructions and suggestions for setting up Gen5 to perform most efficiently and effectively in your lab.

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Set up Gen5

❖ For Gen5™ Only

Gen5™ fulfills the reader control and analytical needs for a wide range of laboratory settings. The degree to which you follow the recommendations provided here depends on the needs of your organization.

Recommended tasks to perform:

1. Designate a **System Administrator**
2. Install Gen5 on the Administrator's computer

❖ Installation instructions are included in the Getting Started Guide shipped with the CD.

3. Change the [System Administrator's Password](#) (see below)
4. Determine the optimal way to store Gen5's protocol and experiment files:
[File Storage](#) (page 4)
 - [Organize the Database](#) (page 10) or your Windows® file structure
5. Install Gen5 for other users and [Connect a Reader](#) (page 21) to each computer
6. If applicable, direct each user's [Database Configuration](#) (page 12) to point to the correct shared database
7. [Set User Preferences](#) (page 24)
8. Learn how to use Gen5:
 - Check out the tutorials (animated demos) in the Help system: Select **Help>Tutorials**
 - Alternatively, begin by performing the Learning Exercises in the Getting Started Guide.

Gen5's System Administrator

For all levels of Gen5 except Gen5 Secure

It is necessary to login as the System Administrator to change User Permissions and Database Configuration, and to access any features which are denied as **Users Permissions**

How to change the System Administrator's password:

❖ This function is only available to the **System Administrator**. You must login, **System> Administrator LogIn**, as the Administrator to access these controls

1. Select **System> User Setup**, and select the Administrator tab
2. Enter the current password in the **Current Password** field. Gen5 ships with the password set to "admin."
3. Enter the new password in both the **New** and **Confirm** password fields
4. Click **OK**.

How to maintain Users Permissions

Except in Gen5 Secure, access to Gen5's functions, like reading a plate, modifying a protocol, and masking values, is defined equally for all users except the System Administrator, who has all "permissions." Learn more on page 5.

Learn about Gen5's Databases

You can opt to save Gen5's protocol and experiment files in Gen5 SharedDB:

- [About Gen5 Databases](#) (refer to the **Managing Files** chapter)
- [Organize Your Database Files](#) (page 10)

File Storage

System > Preferences > File Storage Mode

Use this control to select a method for storing protocol and experiment files.

❖ **Attention Gen5 Secure users:** To ensure 21 CFR Part 11 compliance use the **Gen5 Database** for file storage

About File Storage

Gen5 provides two methods for storing protocol and experiment files. You can use the secure, shared-access database provided with Gen5, which is required for compliance with the FDA's [21 CFR Part 11](#) regulation on electronic records submission.

Alternatively, you can use the file system provided with the Windows® operating system on a local PC or network (LAN). If your organization is unconcerned with FDA regulations, the choice is a matter of preference. However, one advantage to using the Gen5 Database is its ability to recover from a system crash. New and modified files are saved as [Temporary Files](#) in the database and can be used to recover information that wasn't saved before a system failure.

❖ **Clarity™** Luminometer protocol files, with a .bpf extension, cannot be stored in Gen5's shared database. They are typically stored in the C:\Program Files\BioTek\Clarity\protocols folder.

How to

- 🔍 Select an option for storing Experiment and Protocol files:
 - **Gen5 Database:** all actions related to managing and maintaining files, like File>Open, File>Save, Browse..., and so on, will occur in Gen5's SharedDB.
 - **Windows File System:** Gen5 will not control the management of files. Actions related to managing and manipulating files will be determined by the Windows operating system, e.g. you can use Windows® Explorer. Generally during file management activities like File>Open, Windows begins at the last directory and folder used.

❖ **AutoSave Feature:** Gen5 offers this feature to give you additional control over the storage of saved files.

User Setup

❖ For all levels of Gen5 except Gen5 Secure

System > User Setup

Gen5 User Permissions

Only the **Gen5 Secure** product level offers the multiple-user login and password controls required to comply with the FDA's electronic-records submission directive. This page describes the more limited security options provided in other Gen5 products.

About Gen5 User Accounts

There are only two types of Users in Gen5: **Administrator** and **User**, i.e. System Administrator and non-administrator. Neither account be deleted

You Can:	You Cannot:
Change the Administrator's password	Login as a User; only the System Administrator requires login
Change the Permissions for User	Change the Permissions for Administrator: all rights and privileges are already given
Maintain/store Gen5 files in a secure database	Keep track of which user is responsible for Gen5 activities
Select a Startup option and Protocol and Experiment Folders for users	Turn on/off Audit Trail notification. Events for which users are invited to add comments to the change log/audit trail are fixed by Gen5

Permissions

Prerequisite:

To change the User Permissions, you must login, **System>Administrator Login**, as the System Administrator

Function
Add a New Plate: Access to menu options and toolbar buttons for Adding one or multiple plates to an experiment
Delete a plate: Access to Plate menu option to Delete, i.e. remove the plate information and all data associated with the plate (if any) from an experiment
Mask/Unmask values: Access to Mask button in the Plate View to mask the values for selected wells. Masked wells are ignored in data reduction and curve plotting
Edit values: Access to Edit button in the Plate View to change the values of selected wells
Re-read plate: Access to Read button after plate has been read to overwrite the current measurement results with newly acquired measurements
Simulate Read: Access to Simulate option of the Plate Read dialog to let Gen5 simulate a reading instead of actually reading the plate. (Useful for Gen5 training/tutorials.)
Read from File (import): Access to Read From File option of the Plate Reading dialog to acquire/import reading data from a text file
Enter Manually (raw data): Access to Enter Manually option of the Plate Reading dialog to manually enter (type in) reading data instead of actually reading a plate

System Controls

This table describes the capability each Permission gives users.

Function
Manage and Maintain Systems: This switch gives or denies access to the next five items. You can override it by individually assigning access to the permissions
Edit Default Protocol: Access to define or modify the Default Protocol Settings
Edit file storage mode: Access to menu option System>Preferences>File Storage to alter the option: database or Windows® file system

Function
Edit Read from File options:
Manage and Maintain Devices: This switch gives or denies access to the next four related permissions. You can override it by individually assigning access to them
Define Test Plates: Access to Diagnostics options to set up and modify the Universal Test Plate records used to conduct testing
Delete Diagnostic Test History: Ability to delete test records. All users can view the test history, only users with this permission can delete the records
Manage and Maintain File Storage: This switch gives or denies access to the next seven related permissions. You can override it by individually assigning access to them. They are only applicable when File Storage "uses the SharedDB"
Create folder in Database: Ability to create a new folder while maintaining database files and when saving protocol and experiment files. Users denied this function are limited to saving files in existing database folders
Delete/Overwrite folder in Database: Ability to delete or overwrite (Save As) folders and files from/in the database
Export file from Database: When maintaining database files, ability to use the right-click menu to Export to Disk
Rename folder/file in Database: Ability to rename database files and folders in the database
Move folder/file in Database: Ability to relocate folders and files within the database
Import file to Database: Ability to import, paste from clipboard, or drag and drop files from another location
View hidden files and folders in Database: Ability to hide files, to see hidden files, and to reveal hidden files

Set up Gen5 Secure

❖ For Gen5™ Secure Only

Follow these procedures to set up **Gen5 Secure**:

1. Designate a System Administrator
2. Complete the [System Administrator's To Do List](#) (see below)
3. [Organize the Database](#) (page 10)
4. Review/modify [Signature Reasons](#) and other security controls (see Gen5's Help)
5. Define certain **Preferences** by using Gen5's [Default Protocol](#). Each shared database has one Default Protocol which defines the initial settings for all newly-created protocols. (page 24)
6. Set up each user's [Database Configuration](#) to point to the correct shared database, if applicable. (page 13)
7. [Connect a reader to each user's computer](#) (page 21)
8. Advise users to [change their passwords](#) (see Gen5's Help)
9. Encourage users to watch the Help>Tutorials and run through the Learning Exercises provided in the Getting Started Guide.

System Administrator's To Do List

Also see information about the FDA's Requirements on the next page.

Initial Setup Tasks

1. Make sure all designated computers (PCs) and BioTek readers meet the System Requirements
2. **Install Gen5 Secure** on one computer (PC)

❖ Installation instructions are included in the Getting Started Guide shipped with the CD.

3. Start Gen5 and log in as the **System Administrator**
4. Change the System Administrator's password (page 14)
5. Copy the database: Shared.mdb to a secure network location (page 13)
6. Test Database Configuration of the Shared.mdb on the network (page 13)
7. Create/modify User Groups, as needed, and assign User Permissions to the Groups (page 15)
8. Create new user accounts and assign the users to a Group

9. Connect reader(s) to the PC and establish communication (page 21)
10. Repeat steps 2, 3, 6, and 8 for the remaining PCs

Periodic/As Needed Tasks

- Customize the security features to accommodate your organization's needs
- Organize your database files
- Educate users on regulatory requirements and Gen5 best practices
- Establish and implement a procedure and schedule for record retention and archival
- Review records, including any training/user-qualification records

❖ Before modifying a user's account, make sure he/she is not logged into the system. You can check the System Audit Trail to determine who is currently logged in.

FDA's System Administrator Requirements

The FDA's Electronic Signatures Rule (21 CFR Part 11) contains requirements that sites must meet in order to be in compliance. The System Administrator should be cognizant of the following:

- **Administrator:** The site shall select an "Administrator" who will be the person responsible for all high-level administration of the program. This person will control access to the program by adding new users, structuring the individual users authority levels, and reporting to management, as appropriate, on any unauthorized use of the program.
- **Personnel Qualifications:** Personnel who develop, maintain, or use electronic records/electronic signatures shall have the education, training and experience necessary to perform their assigned tasks.
- **Written Policies:** There shall be written policies which hold individuals accountable and responsible for action initiated under their electronic signatures, in order to deter record and signature falsification. There shall be written revision and change control procedures to ensure that the program is administered in compliance with the FDA's requirements.
- **Record Archiving and Deletion:** The site is responsible to ensure that archiving or other file management techniques are suitable such that electronic records generated by the system shall be accurate and readily retrievable. Records may be removed by deleting the entire shared database or individual files within the database. This action may be conducted by the Administrator or by any Power User.
- **User Identity:** Any person who will be authorized to use an electronic signature will have their identity confirmed by the Administrator prior to granting them program access. Only the genuine owner of the electronic

signature is allowed to access the program through their ID/password combination. System users should be informed that accessing the program using someone else's login is a violation of the FDA rule.

- **Password Expiration/Recall:** All passwords must be checked, recalled, or revised at an interval appropriate with the security needs of the organization. Personnel who no longer work at the establishment shall have their program access capabilities deactivated in a timely manner.
- **Certification to FDA:** The site must certify to FDA that the electronic signatures utilized on records are intended to be as legally binding as handwritten signatures, prior to or at the time of record submission to FDA. The FDA rule should be consulted for details and method to be used.
- **Notification of Attempted Security Breaches:** The software utilizes an error log system to notify the Administrator of log-in failure incidents that exceed the limits they have established. The administrator is responsible to “immediately and urgently” notify the appropriate personnel at the site if the activity appears to be an attempt to breach security.
- **Signature Representations:** The administrator should be made aware that while electronic signatures representations cannot be excised or added they can be copied by screen copy techniques and pasted into other documents. These modified documents cannot be re-saved in the secure program but may be printed out as is.

Organize Your Database Files

- ❖ During regular installation, **Gen5 Secure** installs and enables the shared database to store experiment and protocol files. **All other levels of Gen5** must elect to use the database at **System> Preferences> File Storage**

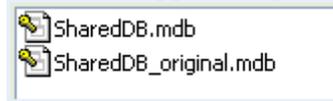
All of your file management requirements can be fulfilled using Gen5's secure databases. You'll be most satisfied with the final structure if you spend some time planning it up-front. In a multiple-user environment, you can set up Gen5's database on a shared-network drive (LAN) so multiple users can access the same protocol and experiment files, including the Default Protocol.

Multiple Databases: You can create multiple copies of the clean, installed SharedDB, renaming them with meaningful titles for use by various projects or teams or researchers. Within each database you can set up a consistent file structure, e.g. specific folders for specific types of Protocols and Experiments, or a different folder for each user. The possibilities are endless.

Backups: Performing backups on a regular schedule is highly recommended to preserve your data. And, Gen5 provides a tool to schedule backups to occur periodically. See below.

File Management Recommendations

- Put a copy of the **SharedDB on a shared-network drive** where all your Gen5 users can access it. Be sure to set each user's [Database Configuration](#) to point to the correct location.
- **Before** moving the SharedDB to a network location, make a copy of it to use as a template for future use:
 1. In the default SharedDB folder, highlight the original, right-click and select Copy
 2. De-select the original (click elsewhere in the dialog), right-click and select Paste
 3. Highlight the copy, right-click and select Rename
 4. Give the copy a unique name, like SharedDB_original.mdb.



- Consider **setting up shared databases** for different projects or teams within your organization. You can follow the steps defined above to create multiple databases in the same folder (or directory), or you can move the unique databases to a different network location/folder. Use Database Configuration to point user's Gen5 sessions to the correct database.
- **Regularly archive and backup** the database to preserve your records. There are numerous ways to do this, so BioTek recommends following your organization's existing policy for securing data. For example, if you put the shared database on the network and your network is backed up every night, this may be sufficient. You can use Gen5's Optimize and Backup Settings to facilitate your data-protection policy.
- Consider using Gen5's automatic [Save](#) feature to create a new, date-stamped folder for storing experiment records. This is an especially good practice for large labs with multiple users who run hundreds of plates per day. Gen5 will keep all that data organized by date. Define this kind of file management setting in the Default Protocol so it will apply to all newly-created protocols.
- Gen5 handles multiple, simultaneous users performing database management tasks by giving precedence to the user with the greater administrative rights.

File Storage

System > Preferences > File Storage Mode

Use this control to select a method for storing protocol and experiment files.

❖ **Attention Gen5 Secure users:** To ensure 21 CFR Part 11 compliance use the **Gen5 Database** for file storage

About File Storage

Gen5 provides two methods for storing protocol and experiment files. You can use the secure, shared-access database provided with Gen5, which is required for compliance with the FDA's [21 CFR Part 11](#) regulation on electronic records submission.

Alternatively, you can use the file system provided with the Windows® operating system on a local PC or network (LAN). If your organization is unconcerned with FDA regulations, the choice is a matter of preference. However, one advantage to using the Gen5 Database is its ability to recover from a system crash. New and modified files are saved as **Temporary Files** in the database and can be used to recover information that wasn't saved before a system failure.

❖ **Clarity™** Luminometer protocol files, with a .bpf extension, cannot be stored in Gen5's shared database. They are typically stored in the C:\Program Files\BioTek\Clarity\protocols folder.

How to

🔍 Select an option for storing Experiment and Protocol files:

- **Gen5 Database:** all actions related to managing and maintaining files, like File>Open, File>Save, Browse..., and so on, will occur in Gen5's SharedDB. Learn [About Gen5's Databases](#)
- **Windows File System:** Gen5 will not control the management of files. Actions related to managing and manipulating files will be determined by the Windows operating system, e.g. you can use Windows® Explorer. Generally during file management activities like File>Open, Windows begins at the last directory and folder used.

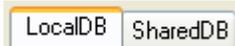
❖ **AutoSave Feature:** Gen5 offers this feature to give you additional control over the storage of saved files.

Database Configuration

System > Database Configuration

Prerequisite

Some features of this dialog require System Administrator privileges.



When Gen5 is installed, there are two primary databases, a shared and a local database. They are named **SharedDB.mdb** and **LocalDB.mdb** respectively. The LocalDB cannot be moved or renamed. The SharedDB can be moved and renamed. All operations affecting them take place using their respective tabs, except the upgrade utility. Learn more: [About Gen5's Databases](#)

Source



Gen5 displays the current location of the database.

- **LocalDB:** You cannot move or rename the LocalDB database, thus the 3-dot button is disabled. If necessary to see the full pathname of its location, you can click inside the text field and scroll to the right.
- **SharedDB:** Click the 3-dot button to view the current location of the database. You can move, rename or copy the **SharedDB** database.

Test



Use the **Test** button to check the connection to the Gen5 database.

- Potential error messages are referenced in the Troubleshooting section above.

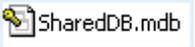
Move/Copy Database to Network

System > Database Configuration

❖ **Gen5 Secure** installs and enables the databases during regular installation. All other levels of Gen5 must elect to use the database to store protocol and experiment files at **System > Preferences > File Storage**

In a multiple-user environment, you can set up Gen5's database on a shared network drive so multiple users can access the same protocol and experiment files. This is a recommended step for Gen5 Secure System Administrators. You can also set up multiple databases, one for each user, for example. During a Gen5 session, access is provided to **only one database at a time**.

How to:

1. Select **System > Database Management > Database Configuration**
2.  Select the SharedDB tab
3.  Next to the Source field, click the 3-dot button
4.  In the Open dialog, highlight and right-click the file SharedDB.mdb, and select Copy or Cut: use cut to move and copy to copy (see File Management Recommendations below)
Note: SharedDB is the installed/original name for the shared database. Since you can change the name, it's possible it has already been changed.
5. Use the browse tools to navigate to the desired location in the **Look in** field
6. When the correct location is selected, right-click in the window and select **Paste**
7. Click the **Open** button to save and close the window and return to the Gen5 Database Configuration dialog
8. Shut down and restart Gen5 to make the changes take effect.

❖ Important: if you're moving the Shared DB to a network drive you may want to consider Disabling Write Caching. Consult your IT department or Microsoft's knowledge base.

Changing the System Administrator's Password

❖ For Gen5™ Secure Only

System > Security > Users

BioTek recommends changing the System Administrator's password immediately following Gen5 installation to ensure a secure operating environment.

To change the password:

1. Login as the System Administrator, if you haven't already done so:
2. Select **System > Login/Logout**
3. Set the **User** to Administrator
4. Enter the default password: **admin**
5. Select **System > Security > Users**
6. Double click the **System Administrator** user (to **Edit** the record)
7. Define and confirm the new password.

❖ Important: Do not forget the Administrator's password. If you do, you'll have to reinstall Gen5.

About User Accounts

❖ For Gen5™ Secure Only

System > Security > Users

Prerequisite

This function is only available to the **System Administrator**.

How to Create, Modify or Delete User Accounts

Only an Administrator can add, modify, or delete users. Except for the Administrator, any user account can be changed or deleted:

-  Click **New** to set up a new user
-  (Double-click or) Highlight a user and click **Edit** to modify its name, password, or Group assignment
- Highlight a user and click **Delete** to remove the user account

Creating/Maintaining User Accounts

❖ For Gen5™ Secure Only

System > Security > Users

Prerequisite

Most options for user accounts are only available to the **System Administrator**. Non-administrators are limited to changing their own password and selecting a Startup Action and Protocol Folder.

User ID

Enter a unique ID using 1 to 16 alphanumeric characters. The user will enter or select this ID when logging into Gen5 and when signing files.

Full Name

Enter the user's name. This name will be associated with events logged by this user's actions and with the digital signature applied by this user.

Group

Choose a Group membership to assign access rights and permissions to the user. Users receive the rights assigned to the Group.

Status

The checkbox shows whether or not the user's account is currently locked. The System Administrator can lock or unlock the account. When a user's account is locked, the user cannot log into Gen5 and cannot sign files. A user's account may become locked due to one of three events:

- Intentional lock by the Administrator through this dialog
- Automatic lock if the user exceeded the number of successive failed login attempts
- Automatic lock if the user's password expired

❖ **Important:** Unlocking a user's account following an automatic lock resets its counter or clock. The reset is specific to the reason for the lockout: when it is caused by password expiration, the password expiration clock is reset and when it is caused by failed logins, the user's history of "successive failed login attempts" is reset to 0.

When lock out occurs due to an expired password, unlocking the account allows the user to login to Gen5 with the same password, giving them a chance to change it. Alternatively, as system administrator, you can simply change the password yourself (which will by default unlock the account) and tell the user to login with the password you have assigned him/her.

Startup Action

Use the drop-down to select the preferred method for starting Gen5:

- **Startup Window** is the default setting, it offers several options including creating a new item or opening a recently used item
- **Create new experiment** opens Gen5 with the Protocol selection dialog open, as if the user had selected File>New Experiment
- **Start at main menu** opens Gen5 showing the File, System and Help menus only. Since neither a protocol nor experiment is open, the workspace is blank.

Protocol and Experiment Folders

Browse to or enter the full path and directory to define the folder in which the current user will typically store protocol and experiment files. If a folder is not specified, Gen5 will default to the most recently accessed folder.

Password

Assign a password for the user to enter the first time he/she logs in to Gen5. Instruct users to change their password after the first login using the Password you've assigned. Users can only change their own password. System Administrators can change any user's password.

Login/Password Controls

❖ For Gen5™ Secure Only

System > Security > Login

Prerequisite

Only the **System Administrator** can access these controls. You must login: System>Login/Logout, as the Administrator to change the settings.

❖ **Important:** The default settings shipped with Gen5 Secure, and shown in the screenshot below, comply with the FDA's 21 CFR Part 11 requirements for controls for identification codes/passwords.

Login

- **Lock user account after:** Specify the number of successive failed login attempts a user may make before being locked out of Gen5. This feature does not apply to System Administrator accounts and only a System Administrator can [reinstate a locked out account](#). Valid entry range: **2-10**. When this feature is unchecked, users login attempts are unlimited. Compliance with 21 CFR Part 11 requires setting a limit for failed login attempts.
- **Lock session after:** Specify the number of minutes that a Gen5 session can be idle before it is locked and requires successful user login to reactivate. A session is considered idle when there is no keyboard or mouse activity and Gen5 is not controlling a reader activity. Valid entry range: **1-1440** minutes. Compliance with 21 CFR Part 11 requires setting an idle-time limit.
- **Force user to type ID:** apply this control if your security rules require users to enter their ID at login and to apply their Signature. When this feature is unchecked, the last user's ID is displayed in the login and signature screens and users can select an ID from a drop-down list of users. This is not a requirement for compliance with 21 CFR Part 11.

Password

- **Minimum password length:** Specify the minimum number of alphanumeric characters required for a valid password. Valid entry range: **2-10** characters.
- **Password expiration:** Specify the number of days a password can be used before users are required to change it. When users let their password expire without changing it, their accounts are locked out and only a System Administrator can [reinstate a locked out account](#). Valid entry range: **1-10000** days. If this feature is unchecked passwords do not expire. Compliance with 21 CFR Part 11 requires an expiration period.

Lock out: when a user's password has expired, the system administrator has two choices:

- manually remove the Locked out flag: this resets the password expiration period allowing the user to login using his/her current password.
- enter a new password for the user (which unlocks the account) and tell the user to login with the password you have assigned him/her. Advise the user to change the password after logging in.
- **Advise user:** If password expiration is set, specify the number of days before their password expires to alert users to change their password. Valid entry range: **1-30** days, but cannot exceed the number of days to Password Expiration.
- **Password reuse:** Specify the number of passwords Gen5 will remember for each user's account to prevent a recently used password from being reused. Valid entry range: **2-20**.

About User Groups

❖ For Gen5™ Secure Only

System > Security > Groups

Prerequisite

This function is only available to the **System Administrator**.

Gen5 Secure uses Groups to manage the rights or permissions granted to users. When creating (or maintaining) a group, you define the level of access and the controls available to certain types of users, and then assign actual users to the groups. Gen5 ships with three groups: Administrator, Power User, and Standard User.

The System Administrator and Power User groups are given access rights to all functions. The Administrator's rights cannot be changed, and include additional rights to manage user accounts that are not extended to Power Users. When Gen5 Secure is installed, the Standard User is limited to the following permissions. The System Administrator can change these controls as needed:

- Quick Read/Use Default Protocol
- Add a New Plate
- Create/Edit Sample IDs
- Edit Plate Information
- Edit Report Builder
- Create folder in database

How to create new and modify existing groups:

Only a System Administrator can add, modify, or delete groups. Except for the Administrator group, any group can be changed or deleted, and any group can be renamed.

-  Click **New** to set up a new group
-  Highlight a group and click **Edit** to modify its name and permissions
-  Highlight a group and click **Delete** to remove it as an option. First you must reassign any users to another group. You cannot delete a group with users assigned to it.

Set up Gen5 Reader Control

❖ For Gen5™ Reader Control Only

Recommended tasks to perform:

1. Install Gen5 on the computer

❖ Installation instructions are included in the Getting Started Guide shipped with the CD.

2. Determine the optimal way to store Gen5's protocol and experiment files:
[File Storage](#) (page 4)
3. [Connect a Reader](#) to the computer (page 21)
4. [Set User Preferences](#) (page 24)
5. Learn how to use Gen5:
 - Watch the online demos, select **Help>Tutorials**
 - Alternatively, begin by performing the Learning Exercises in the Getting Started Guide.

Connecting a Reader

System > Reader Configuration

After following the Operator's Manual instructions for attaching the reader to the computer, you must tell Gen5 what type of reader it is and which communications port (Com Port) it is plugged into. Gen5 and Gen5 Secure allow up to two readers to be assigned at a time.

❖ **Special note for Clarity users:** Configuration parameters and port settings can only be defined through the Clarity PC software: Follow instructions on page 23.

1. Turn on the reader
2. From the menu, select **System > Reader Configuration**
3.  Click the **Add** button to define the Reader Settings
4.  Use the drop-down list to select the **Reader Type**
5. Except for the [Clarity](#) (see page 23), in the **Com Port** text field, enter the number of the communications port.
6. Retain the default **Baud Rate**.
7. Click **Test Comm**. Gen5 will attempt to communicate with the reader.
8. After you receive a passing message, "The reader is communicating," click **OK** and then click **Close** at Reader Configuration. If you receive any other message look for a remedy in the **Troubleshooting** section of this guide.

That's it! Gen5 captures the information it needs from the reader itself, including probe size, wavelength and bandwidth capability, and any other applicable information.

Instrument-Specific Information

- **Synergy™ HT:** For this multi-detection reader, Gen5 must obtain the fluorescence/luminescence filter sets. BioTek sets the reader's on-board software with the ordered configuration before shipping the reader. Gen5 will capture the stored information when it initiates communication with the reader.
- **Synergy™ 2:** For this multiple-module, multiple-detection reader, Gen5 must obtain both the fluorescence/luminescence filter sets and the mirror-holder configuration. BioTek sets the reader's on-board software with the ordered configuration before shipping the reader. Gen5 will capture the stored information when it initiates communication with the reader.
- **Clarity™:** A Clarity luminometer can be added or deleted in the instrument configuration dialog. But, unlike other readers, the COM port that is used to attach to the Clarity **cannot** be set or modified in Gen5's Reader Configuration

dialog. You must use the Clarity software to define the connection settings. Use the **Clarity Control Panel**.

- **FLx800™**: Gen5 must obtain the fluorescence/luminescence filter wheel configuration. BioTek sets the reader's on-board software with the ordered configuration before shipping the reader. Gen5 will capture the stored information when it initiates communication with the reader.
- **PowerWave™**: This 8-channel monochromator does not need special set up. When Gen5 communicates with the reader the Absorbance Wavelength table currently stored in the reader's memory is displayed. When defining a Read Step select from the stored wavelengths or enter a different one.
- **PowerWave™ XS**: This single-channel monochromator does not need special set up. When Gen5 communicates with the reader the [Absorbance Wavelength](#) table currently stored in the reader's memory is displayed. When defining a Read Step select from the stored wavelengths or enter a different one.

❖ Two models of PowerWave XS are listed in the Gen5: **PowerWave XS** and **PowerWave XS2**. If you are connecting a PowerWave XS reader that has a USB port and an MQX200R2 product number (take note of the 2), you must select the PowerWave XS2. Our changes to the PowerWave XS hardware to incorporate a USB/RS-232 com port requires unique reader identification in Gen5. There is no difference in the optical performance characteristics of the reader.

- **ELx800™** and **ELx808™**: BioTek configures the on-board software of these filter-wheel-based readers with the installed filters. Gen5 will capture the filter configuration when it initiates communication with the reader.
- **µQuant™**: This single-channel monochromator does not need special set up. When Gen5 communicates with the reader the Absorbance Wavelength table currently stored in the reader's memory is displayed. When defining a Read Step select from the stored wavelengths or enter a different one.

❖ Learn about the Absorbance Wavelengths tables in the Reader Control and Configuration chapter.

Setting up the Clarity Luminometer

Clarity users must follow a slightly different sequence of steps to establish communication between the Clarity and Gen5. First, follow the installation instructions provided with the Clarity, including installing the Clarity PC software. After installation, when you're running assays, Gen5 uses the Clarity PC software in place of its **StepWise™ Procedure**. But, you define the other elements of the Protocol and run the Experiment with Gen5.

❖ **Important:** Install the Clarity PC software before proceeding.

1. Connect the luminometer to the computer (if not already connected) and turn it on.
2. Start the Clarity software and set up the communications port (Options> Com Port Settings).
3. Then, select **Options> Instrument Info** to test communication. Details about the current instrument should be displayed on-screen. If not, repeat Step 2, making sure the correct port is selected.
4. **Important:** Close the Clarity software.
5. Start Gen5 and log in (if required).
6. Select **System> Reader Configuration** and set the **Reader Type** to **Clarity**
7. Select **System> Reader Control> Clarity** to make sure Gen5 is communicating with the Clarity. If the control panel does not open, repeat Step 6 and retry. Contact BioTek TAC if problems persist.

If the control panel does not open:

- on Windows® XP and 2000 systems, repeat Step 6 and retry.
- on Windows® **Vista** systems:
 - 1 Locate and right-click the **Clarity** desktop icon, and select **Run as administrator**. If prompted, enter the password,
 - 2 At the **User Account Control** dialog, click **Allow**,
 - 3 The Clarity software will launch and communicate with the reader. **Close** the Clarity software.
 - 4 Return to Gen5 and test communication with the Clarity by performing Step 7 above.
- Contact BioTek TAC if problems persist.

Setting up Preferences

Gen5™ offers tools for defining certain preferences. Take advantage of them to save time and enforce consistency of use. They can be overridden, as needed, during normal use of the software.

Set up the Default Protocol

The Default Protocol can be used to define numerous settings that you're likely to apply to all experiments. Consider using the Default Protocol to:

- [Customize Well IDs in the Plate Layout](#)
- Set up Protocol Options to automatically save files and to automatically execute your preferred method of results output immediately after reading a plate. Define File Naming Conventions and File Locations for your export files under Export Options

Set Startup Preferences

Set user's **Startup Preference** and **Protocol and Experiment Folders**:

- Gen5 Secure: [Creating/Maintaining User Accounts](#) (page 15)
- All other Gen5 levels select **System>User Setup**

Define Startup Preferences

1.  Use the drop-down to select the preferred method for **Startup Action**:
 - **Display Welcome dialog** is the default setting, it opens Gen5 with a screen that offers links to several common tasks including creating a new item or opening a recently used item. Note: the only way to access the Welcom page is to launch Gen5.
 - **Create new experiment** opens Gen5 with the Protocol selection dialog open, as if the user had selected File>New Experiment
 - **Start at main menu** opens Gen5 showing the File, System and Help menus only. Since neither a protocol nor experiment is open, the workspace is blank.
2.  Use the 3-dot button to change your **Protocol** and **Experiment Folders**: browse to the full path and directory to define the folder where you will typically store protocol and experiment files. Gen5 will point to these folders when you save and open a protocol or experiment.
3. Click **OK**.

The changes will take effect the next time you log into Gen5.

Customize the Toolbar

Gen5 has two toolbars, one for each mode: Protocol or Experiment. You can change their configuration to facilitate your work. Find instructions in the System Management chapter.

Select a List Separator for Import Files

If you regularly import data files, rather than obtaining measurements from the reader, take a moment to identify for Gen5 your normal list separator. Select **System>Preferences> Read from File Settings**

Chapter 2

Getting Started

This section provides a basic introduction to Gen5. It also includes contact information for obtaining technical support and tips for users of BioTek's KC4 software.

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Getting Started

Welcome to Gen5™! To make you feel comfortable with this very capable reader control and data analysis software, we've provided several learning tools. Tackle them progressively and you'll be a pro in no time. You'll find these tools in Gen5's Help.

❖ **Important:** these topics do not describe the [Initial Setup](#) requirements, like connecting a reader. Unless your System Administrator has already done so, complete the initial setup requirements before trying the lessons described here.

● Watch an Online Demonstration: select Help > Tutorials

- Basic #1: Intro to Gen5
- Basic #2: Setting up the Procedure
- Basic #3: Defining the Plate Layout ([not for Gen5 Reader Control](#))
- Basic #4: Creating Data Reduction Steps ([not for Gen5 Reader Control](#))
- Basic #5: Runtime Prompts
- Basic #6: Building Reports
- Data Views: Create a New View
- Intro to Power Export (to use Microsoft® Excel for reporting) ([not for Gen5 ELISA or Reader Control](#))
- How to create multiple standard curves
- Intro to Calibrator-Plate Protocols ([not for Gen5 Reader Control](#))
- How to select the optimal PMT Sensitivity (for fluorescence and luminescence)
- Using the Synergy 2: Fluorescence Detection Review

● Practice using Gen5 with the Learning Exercises:

- Refer to the Getting Started Guide shipped with the product CD or Gen5's Help system for step-by-step learning exercises

● Open a Sample Protocol:

Gen5 ships with numerous protocols so you can load one up, customize it to fit your particular requirements, and run an experiment.

1. Select **File>Open Protocol**
2.  At the top of the Open dialog, click the "Up One Level" button
3. In the Gen5 folder or the DB (shared database), select the **Samples** folder
4. Select the folder for the desired detection method: **Absorbance**, **Fluorescence** or **Luminescence** or for **Synergy 2** users, there is a **Synergy 2** folder within each detection method folder
5. Open the **Protocol** folder and select one

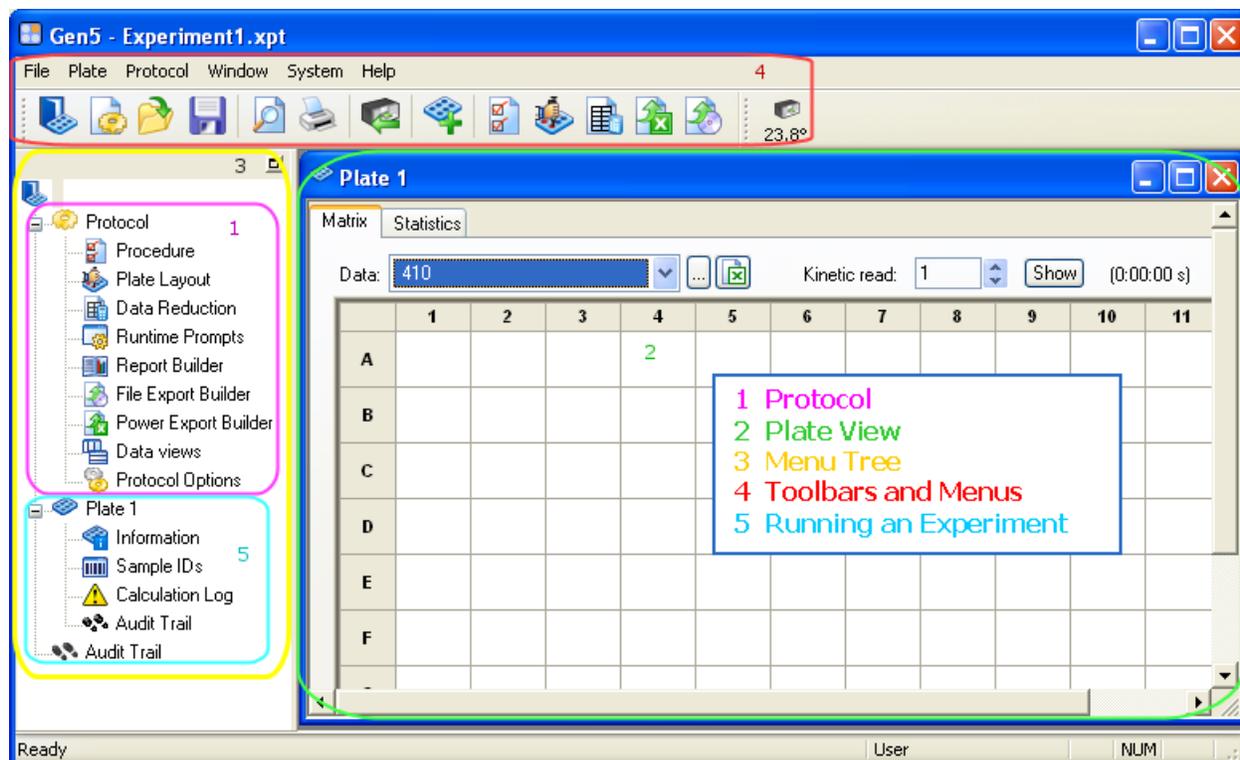
❖ **Recommendation:** Before making any modifications to the sample protocols, we recommend selecting File>Save As after opening them and assigning a unique name to the protocol. This will preserve the original sample protocol for future use. Also recommended when saving a copy of the original protocol is selecting the most convenient location for it. You may want to use the Up One Level button a few times to get back to the Gen5/Protocol folder, a more likely location for your "in use" protocols. Note: the step-by-step instructions for opening a sample protocol are based on the default filenames and path.

❖ **Important:** The sample protocols must be considered as examples provided for demonstration and guidance purposes. If you plan to use these protocols or similar ones in a real application, it is your responsibility to validate the protocol parameters, including the report and export content (if applicable), before using them.

❖ **One more thing:** your reader may not support all of the sample protocols provided. Review the descriptions in the Sample Protocols and Experiments Guide to see if your reader is compatible with the defined steps. If you can read PDF files: click the Windows® **Start** button, select **All Programs>Gen5>Sample Protocols and Experiments Guide**

Gen5's Workspace

Gen5 offers several controls and workspaces for developing protocols, running experiments, and viewing and reporting results:



❖ Gen5's **Welcome** screen is only available when Gen5 is initially launched. You restart Gen5 to get to the Welcome screen.

1 Protocol

Every experiment is based on a protocol. The differences between a Protocol and an Experiment in Gen5 are described in the Essential Concepts chapter.

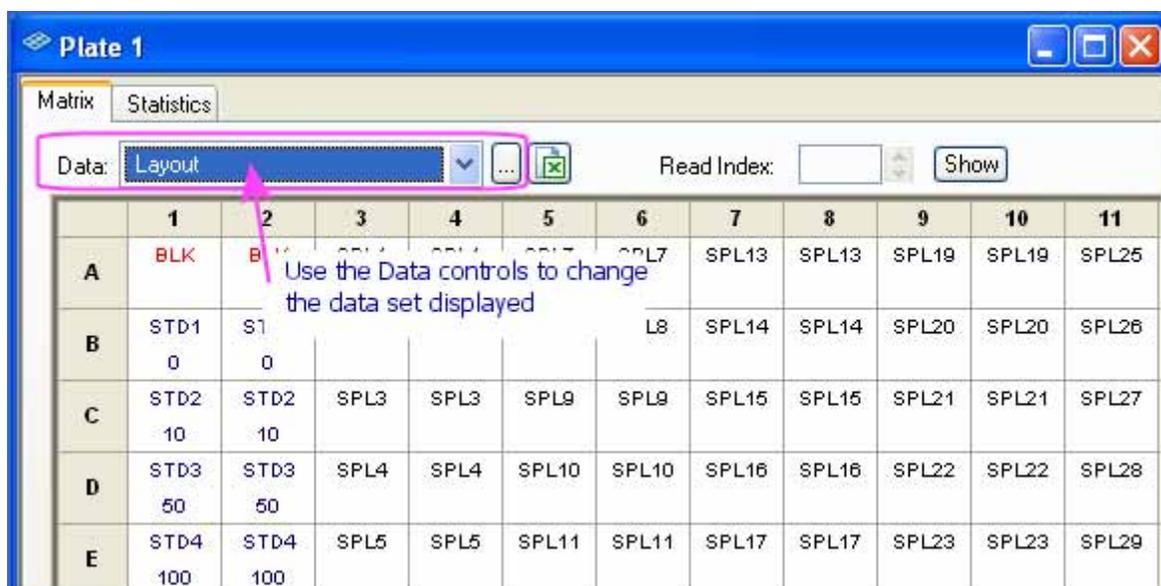
2 Plate View

Gen5 provides a view or workspace for each plate processed (or to be processed) in an Experiment. You must have an Experiment, rather than a Protocol, open to have a Plate View:

Opening the Plate View/Workspace

In an [Experiment](#), if it is not already open in the main view of Gen5™:

- from the menu tree: Double-click the desired  Plate 1 item
- Or, select **Plate > View**



Gen5 offers several ways to modify and customize the Plate View for on-screen display and reporting/outputting results, see the [Viewing Results](#) chapter to learn more.

3 Menu Tree

The menu tree, docked at the left side of the workspace, provides a quick and easy way to open all the tools needed to create and run experiments.

- [About the Menu Tree](#) (page 38)

4 Toolbars and Menus

Here is a reference guide to learn about Gen5's buttons and icons:

- [Buttons and Icon Guide](#) (page 36)

5 Running an Experiment

All of the views and components come into play when running an experiment (File>New Experiment). The Protocol menu tree is the primary engine for an experiment and Plates (one for each plate processed or to be processed) are added to the menu tree. The Plate View and other plate components are made available:

 **Information** contains the text input at the Runtime Prompts when the plate is read and Bio-Cell™ results when this pathlength-correction option is used

 **Sample IDs** are user-defined sample names or bar codes

 **Calculation Log** keeps track of and displays any data reduction errors

 **Audit Trail** logs changes (masking and editing) of data points for all levels of Gen5, and numerous other events for Gen5 Secure. Audit trail events can be included in reports and export files.

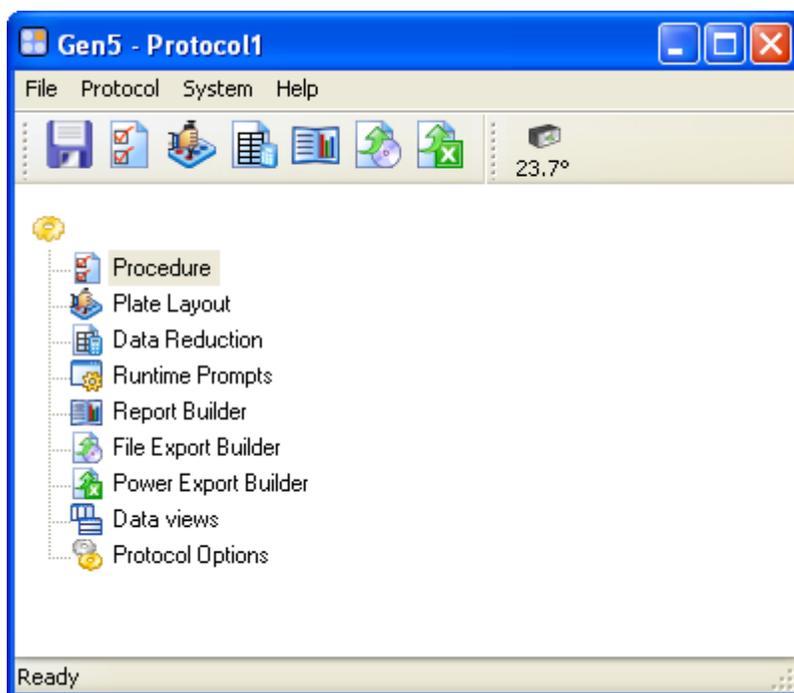
About the Plate Workspace

The **Plate View** represents the microplates processed (or to be processed) in an **Experiment**. The following tabs are available depending on the Protocol definition:

- **Matrix:** a representation of the microplate layout. Each cell in the grid shows the data-set results for the well it represents in the plate. The Layout data set shows the current [Plate Layout](#). You can use this view for [masking or editing results](#).
- **Statistics:** a standard set of statistical values are determined for you and displayed in a table
- **Graphs:** when a [standard curve](#) is defined it is shown in the Graphs tab
- **Cutoff Values:** when a [Cutoff](#) Data Reduction step has been defined, this tab displays the values or results of the cutoff formulas
- **Validation Results:** when a [Validation](#) Data Reduction step has been defined, this tab displays the results of any calculations

Introducing the Protocol Workspace

When you create a new protocol, Gen5 opens a special workspace limited to the protocol's components:



The workspace is made up of the menu tree with a branch for each of the protocol's elements. The order of the protocol elements reflects the order to follow when defining most protocols:

 Defining the **Procedure** or reading parameters, like detection method, wavelength, and other factors, is the most important step to Gen5. The **Procedure** describes the data sets which are used in most subsequent steps to generate results output. The **Plate Layout** is the only other protocol element that is not affected by the Procedure.

 For most protocols, it's best to define the **Plate Layout** in your second step. Gen5 automatically performs a blank-subtraction calculation when Blanks are defined in the plate layout. (You'll see this Transformation in the Data Reduction workspace.) Defining the standards and their concentrations in the plate layout is a prerequisite to generating a standard curve.

 **Data Reduction** is one of Gen5's most powerful features, and it requires the information provided by the two previous steps to logically offer you its capabilities. Automatically-generated transformations, like pathlength correction, and the ability to conduct well analysis, for example, depend on the **Procedure**. To plot a standard or titer curve, and to validate Transformation formulas requires knowing the **Plate Layout**.



Runtime Prompts are user-defined text fields that are presented to users when they read a plate. The information obtained is stored in the Plate Information component of the experiment and can be included in reports and export files.



Viewing and Reporting Results: the next four options (or three options if you're running Gen5 ELISA or Reader Control, which do not offer Power Export) are tools for selecting and customizing the appearance of data sets: raw data measurements and data reduction results. **Data Views** controls the on-screen appearance of data. Data Views also stores and makes available for reporting and exporting any customizations made to the data sets. For example, you can build your own matrix, table, or curve view of a data set by combining and formatting data elements. When you do this in Data Views, that user-defined data set is available for printing or exporting using the **Report Builder**, **File Export Builder** and **Power Export Builder**.



Protocol Options provide several special features and preferences. Many of the options may be rarely used by your organization. Review the options provided and if your preferences vary from the default settings, use the **Default Protocol** to set them for all newly-created protocols. The exception to this rule occurs with multi-plate protocols: Calibrator-Plate Protocols and, for Gen5 and Gen5 Secure users, multi-plate assay protocols. These types of protocols begin by selecting the Protocol Type defined in the Protocol Options.

Gen5's Wizard

Use Gen5's Wizard to create a new protocol. Then, you can run an unlimited number of experiments based on that protocol. Learn the difference between a [Protocol and Experiment](#) in Gen5, in the Essential Concepts chapter to make the most of Gen5.

How to use the wizard:

1. On each screen of the wizard, click the button to define that protocol element (each function is described in a subsequent chapter):



Defining the **Reading Procedure**



Defining the **Plate Layout**



Setting up **Data Reduction**



Building Reports



Using the **File Export Builder**



How to use **Power Export**

2. After defining a protocol element, click **Next** to proceed to the next one. Gen5 displays a checkmark for an element that has been defined.
3. Click **Finish** to generate the protocol based on your selections.
4. Select **File>Save** and give the protocol a unique name. Now you're ready to run an experiment.
5. Select **File>New Experiment**. The just-created protocol will be highlighted, click **OK** to select it.

Buttons and Icons Guide

Buttons	Descriptions
	The 3-dot (three-dot) button is a tool for customizing or modifying the item's contents. In some report options, you must click in a field to activate its 3-dot button
	Open an existing Experiment
	Create a new Experiment or Protocol
	Add another plate to the current Experiment
	Read the plate (or Simulate, Manually Enter, or Import data)
	Save the Experiment
	Gen5's Wizard to help you create an Experiment
	Print the results, but first use Report Builder to select what to include
	Print Preview
	Reader Control: check the status, open the control panel
	Duplicate View: click the button in the upper-right corner of the Plate View to open a coincident display of the plate's results
	Gen5 Secure Only: Sign a protocol or experiment

Menu Tree Icons	
	Plate - Not read. Put the plate in the reader and click the Read button
	Plate read successful
	Plate read paused by Stop/Resume step. When you're ready, put the plate in the reader, click the Read button and select Resume to continue
	Plate read aborted. To begin again, put the plate in the reader, click the Read button and select Re-Read
	Plate read in progress
	Plate read error, which is always preceded by an error message

	Protocol
	Procedure: define the reading parameters
	Plate Layout: assign location of samples
	Data Reduction: set up calculations
	Runtime Prompts: define the information requested when a plate is read
	Report Builder: select the content to print
	File Export Builder: select the content to export
	Power Export Builder: select the content to export to Excel®
	Data Views: customize the appearance of data for online viewing and reporting
	Protocol Options: miscellaneous options for saving, naming, exporting and calculating results
	Plate Information: information obtained at runtime
	Sample IDs: user-defined names or IDs assigned to samples
	Calculation Warning Log: Data Reduction-related errors issued by unexpected curve or calculation results
	Multi-Plate plate view of data reduction statistics and curves
	Audit Trail displays any logged events

3-dot (Edit) Button

The 3-dot button leads to editing features for the field or data point it is associated with. Click the button when it is next to or in a field to change the selection list or format of the field's items.

❖ **Note:** In [Field Groups](#) and in [Headers and Footers](#) you must click inside a field in the table to enable a 3-dot button.

About the Menu Tree

- In an experiment, the menu tree is docked at the left side of the workspace, unless its position has been previously altered. When you're working with a protocol file, the menu tree, like the toolbar, is limited to related operations. [Learn the difference between Gen5's Protocols and Experiments](#) in the next chapter
- The menu tree provides a visual cue of the steps to follow when creating a protocol
- All of the controls available from the menu tree can alternatively be accessed using toolbar buttons or the drop-down menus
-  and  icons next to an item expand or close it to reveal or hide its components
- Highlight an item in the menu tree and **right click** for a context-sensitive menu of options, including **Read** when a plate is selected, for example.
- * asterisks are displayed next to plate icons (and in the title bar) of an experiment when a change is made or an action is taken but the file has not yet been saved
-  You can move the menu tree to another corner of the workspace or let it float undocked like the Plate workspace: click the undock button, drag the title bar and drop it in the desired location
- When you **Add** multiple plates to an experiment, highlight a plate and right-click for menu options to **delete** and **renumber** plates.

Tips for KC4 Users

BioTek relied on input from KC4 users to develop this improved next-generation product. It may take a bit of practice to learn how to use them, but we think you'll find the new features worth it.

1. In Gen5, **reading parameters** are not defined in one dialog (screen), but set up as steps in the **StepWise™ Procedure**. This gives you far more flexibility in defining an experiment. Depending on the level of Gen5 you're running, this includes multiple read steps and reading-related functions, like shaking and dispensing.
2. The **filename extensions** are slightly different and file formats are simpler. Gen5 replaces the .pla and .glb formats with one experiment file: .xpt. Gen5 keeps the .prt filename for protocol files. The experiment file (.xpt) like its predecessor the .glb or global data file, contains the .prt or protocol as it was defined at runtime

KC4	Gen5
.pla .glb	.xpt
.prt	.prt

3. Gen5 does not offer **Power Reports** for Microsoft® Word. Power Export to Excel is available and a full-featured, user-customizable **Report Builder** eliminates the need for Word for most users. In addition, a new **Quick Export** to Excel feature and a user-customizable **File Export** tool are offered, which can be used to port files to Word. Learn more: [Reporting Results](#)
4. **Append to Kinetic File**, a KC4 feature, has been replaced in Gen5 with new features:
 - multiple read steps can be performed in one experiment, and Gen5 offers to **Append to previous Kinetic data** when multiple kinetic loops are defined in a Procedure
 - a long, discontinuous-interval Kinetic analysis can be performed using the [Discontinuous Kinetic](#) option
 - multiple plates can be used to run a single assay, review [Multi-Plate Protocols](#)
5. **Eject Between Filter Sets**, a KC4 option, is omitted from Gen5 because it is not needed. Instead, you can set up multiple read steps interspersed with a Plate In/Out or Stop/Resume step
6. **Lag time** defined in Reading Parameters in KC4 is accomplished with a **Delay** step in the Procedures in Gen5

7. In Gen5, add a **Kinetic** loop to the Procedure rather than ticking a checkbox in KC4. See [Setting up a Kinetic Analysis](#). Also note that the Data Reduction options for kinetic analysis do not include KC4's "Formula" and individual reading point identifier "R." These calculations can be replicated using Gen5's Transformation dialog.
8. In Gen5, to "**Pre-Read Blank Plate**" you'll set up two Read Steps with a Stop/Resume step between them: for instructions see [Subtracting a Blank Plate](#) (This is not available for Gen5 ELISA)
9. Gen5 does not automatically generate dual-wavelength subtraction or **Delta OD** data reductions, for instructions see: [How to perform Dual-Wavelength Subtraction](#)
10. Set up a **Dispense** step in the Procedures, rather than ticking a checkbox in KC4. Here are instructions for setting up [Dispensing Protocols](#)
11. **Luminescence reading** parameters in Gen5 require definition of **Integration Time** rather than the number of samples per well and delay time before and after sampling. When selecting the Emission filter setting, **Lum/E** has been replaced with **Hole**.
12. Don't confuse what was called "**Multi-Plate Transformations**" in KC4 with multi-plate protocols or multiple-plate experiments in Gen5. Multi-plate transformations have been replaced with the **StepWise™ Data Reduction** steps, where an almost unlimited number of calculations can be performed
13. [Multi-Detection](#) was called Multi-Mode in KC4
14. Raw Data Correction options in KC4 are engaged differently in Gen5:
 - Blank Plate Subtraction is described above
 - [Blank Wells Subtraction](#) occurs automatically when there are Blanks assigned to the Plate Layout. You'll find these automatically-generated Transformations in the **Data Reduction** dialog
 - **Pathlength Correction** can be enabled when defining an Absorbance Read Step
15. When viewing **Well Analysis** results in the Well Zoom view:
 - Gen5 uses brackets [] to show the revised Calculation Zone, instead of showing the data points in different colors as in KC4
 - Setting "**Scales for Kinetic Reads**" and "**Individual Well Auto Scaling**" for scanning reads is replaced with more feature-rich tools for editing the results output, see [Modify a Graph](#). When you combine these features with the ability to define the calculation zone (select Calculation Options when defining the Well Analysis step), you have enormous control over the appearance of the results
16. [Monitoring Wells](#) is more flexible in Gen5 because it is defined as a separate step in the Procedure. The read parameters are specific to the monitoring process and can be different than those defined to obtain measurements

Getting Technical Assistance

Gen5™ is backed by a superior support staff. If the software fails to work perfectly, please contact BioTek's Technical Assistance Center (TAC). You can call, write, fax, or email your questions and concerns to BioTek:

Email Support: *tac@biotek.com*

Fax Support

Send a fax with your questions or requests for help 24 hours a day:

- Technical Assistance Center (TAC): 802.655.3399
- European Coordination Center: +49 (0) 7136.968.111

Phone Support:

You can telephone the Technical Assistance Center between 8:30 AM and 5:30 PM Eastern Standard Time (EST), Monday through Friday, excluding holidays.

Customer Service: (802) 655-4040

Technical Assistance Center:

- In the US call: (800) 242.4685
- Outside the U.S. call: (802) 655.4740

European Coordination Center: +49 (0) 7136.9680

Whichever method of contact you choose, please be prepared to provide the following information:

- The software version and revision numbers displayed at **Help>About Gen5...**
- The license type or software level: Gen5, Gen5 Secure, Gen5 ELISA, or Reader Control
- The specific steps that produce your problem
- Any error codes displayed
- A daytime phone number
- Your name and company information
- An email address and/or a fax number, if available.

Chapter 3

Essential Concepts

This section reveals the basic concepts upon which Gen5 was built. Learning them will enhance your experience using Gen5.

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File Storage	47
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Essential Concepts

Understanding the basic concepts behind Gen5's structure and behavior will help you make the most of it. A few topics are covered here, on the next few pages, more information is provided in Gen5's Help.

- Experiment vs. Protocol on page 45
- **File Formats:**
Gen5's files are identified by their filename extension:
 - .prt = Protocol file
 - .xpt = Experiment file (contains the protocol and any data acquired or generated within the experiment)
- File Storage on page 47
- Best Practices on page 48

In Gen5, select Help>Help Topics to find:

- **Multiple-Plate Experiments**
- **Security and FDA Electronic Records Compliance**

❖ Only the **Gen5 Secure** level of software offers all the capability required to meet the FDA's electronic records requirements: §21 CFR Part 11.

Experiment vs. Protocol

Gen5™ uses two common terms to define distinct elements of its toolkit. The distinction is subtle and understanding it will improve your Gen5 experience.

Protocol (*.prt)	Experiment (*.xpt)
A protocol is a "recipe" or set of instructions designed to capture, transform and report and/or export data	An experiment has a copy of the protocol and at least one plate. It executes the instructions provided by the protocol to produce results
Protocols are created and saved as standalone files. They function as a template; an unlimited number of experiments can be based on one	While an experiment is created using an existing protocol, the experiment's copy of the protocol can be modified within the experiment
A protocol consists of reading requirements, like detection method and wavelength, and reading-related actions, like shaking and incubation (Procedure), plate layout, data reduction, and data viewing, reporting and exporting definitions	Running an experiment is the only way to process a protocol. Gen5's Quick Read function may at first appear to skip the protocol development stage, but it uses the default protocol, and generally requires reading parameters to be defined
A protocol can be used repeatedly (as-is or modified) within experiments. By itself, a protocol does not produce results. Protocols do not have plates associated with them	Multiple plates can be processed in an experiment; each one considered a unique assay with independently reported or exported results. The exception is multi-plate protocols, described later
.prt is the protocol's filename extension	.xpt is the experiment's filename extension
A copy of the protocol is saved within an experiment, or as a standalone .prt file. Since protocols do not have plates, they cannot generate data outside of an experiment	An experiment is saved as the full collection of procedures, formulas, reporting definitions, and other details, i.e., the protocol, and the plate data from readings and calculation results
The Gen5™ Secure level of software maintains an audit trail of all activity and changes related to a protocol. All other Gen5™ software levels do not support this feature	Data acquired and transformed in an experiment is protected by an audit trail in both Gen5 Secure and other Gen5 software editions. The Reader Control edition does not support this feature

Protocol (*.prt)	Experiment (*.xpt)
Changes made to a standalone protocol are not reflected in any previously created experiments based on that protocol. A new experiment must be created to apply the revised protocol	Within an experiment, you can select Save Protocol As to capture the current details of the protocol and save them as either a new protocol or as an overwrite of the original protocol

- ❖ All newly created protocols and (unless another protocol is selected) experiments are based upon the [Default Protocol](#)
- ❖ Gen5™ also supports more complex multi-plate protocols that are not covered in this introductory material. Check out: [Designing a Multi-Plate Protocol](#) in a subsequent chapter.

About File Storage

File Types

Gen5™ creates two file types: Protocol = .prt and Experiment = .xpt

- The Gen5 executable file (.exe) and numerous other types of supporting files, like an Excel® template, are also installed on the computer.
- In addition, **Clarity™ Microplate Luminometer** users will work with Clarity protocol files, which use a .BPF extension. Gen5 references the Clarity files as they contain the reading parameters required to control the luminometer.

Databases

Gen5 installs two databases on your system called LocalDB and SharedDB. While the databases are always used for critical, internally-used files, Gen5 offers you the choice of using the Windows® File System or the Gen5 (SharedDB) database for storing Gen5's Protocol (.prt) and Experiment (.xpt) files. This option, combined with the ability to create multiple databases, allows you to structure file storage according to your organization's requirements.

- Files may be stored on the computer's hard drive, on a network, or on a CD or other portable medium. Windows Explorer or a similar application can be used to view the file names and locations, and to move, copy, rename, and delete files.
- Alternatively, protocol and experiment files may be stored in a secure, shared-access database. This database, initially named SharedDB.mdb, can be stored on a user's computer or on a shared-access network/computer (LAN). Gen5 provides a special file maintenance utility for viewing the file names and their locations, and for moving, copying, renaming, deleting, importing, and exporting files.
- Select the preferred method of storing protocol and experiment files at **System>Preferences>File Storage**

File Location

During a conventional installation:

- the program files are stored in this default location: C:\Program Files\BioTek\Gen5 (*software edition*)
- the databases are stored in these default locations:
Windows XP and 2000 systems: C:\Documents and Settings\All Users\Application Data\BioTek Instruments\Gen5 (*software edition*)*(version #)*\SharedDB or LocalDB
Windows Vista: Windows XP and 2000 operating systems: C:\Program Data\BioTek \Gen5 (*software edition*)*(version #)*\SharedDB or LocalDB
- Gen5 installs Protocol and Experiment folders in the respective File Storage locations, e.g. C:\Program Files\BioTek\Gen5 (*software edition*)\Protocol

Best Practices

Like most software tools, Gen5™ is flexible, it offers several ways to accomplish a task. Here are some recommendations for saving time and using it most efficiently:

Efficiencies

- For an assay or experiment that you will run numerous times, develop a **Protocol** to define the Procedure, Data Reduction, Data Views and Reports required. Then you can run an Experiment (File>New Experiment) based on the Protocol whenever necessary. You can fine-tune the protocol within an experiment, but remember to select File>Save Protocol As to update the original protocol with your improvements.
- Just like word processing documents, when you run similar types of experiments, you can use File>Save As to give you a head start creating a new protocol based on an existing protocol that contains the same plate layout, reading parameters, or other elements that will be repeated in your new protocol.
- Define and customize [Data Views](#) before selecting what to include in reports or export files. All the on-screen data (i.e. data views) can be reported or exported. If you use on-screen views and paper reports equally, it is most efficient to first fine-tune the Data Views, and then include them in reports/exports.
- Always assign **Blanks** to the plate. Blanks can be deionized (DI) water, buffer, reagent without analyte, substrate and so on. When running fluorescence cellular assays, a DI-water blank illustrates the background contributed by the instrument and labware as separate from the cells and media. Identify the location of the Blanks in the [Plate Layout](#) and Gen5 will automatically create the blank-subtraction data reductions.
- Backup your database regularly, BioTek recommends once per week for most organizations. If you're using Gen5's Database for protocol and experiment file storage, use the built-in Periodic Optimization feature.
- Take action if you get a warning message about the remaining size of your databases, see [Maintaining Files](#) for instructions on reducing the database size.
- Consider using Gen5's automatic [Save](#) feature to create a new, date-stamped folder for storing experiment records. This is an especially good practice for large labs with multiple users who run hundreds of plates per day. Gen5 will keep all that data organized by date. Define this kind of file management setting in the Default Protocol so it will apply to all newly created protocols.
- Turn off the Multi-Read Calculation option to improve Gen5's performance. Calculation results will be the same, but your PC's resources will not be diverted for performing interim calculations.

Time Savers

- **Partial Plate:** for assays using strips or partially-filled plates, especially if the read steps are long or complicated, you can save time by telling the reader exactly which wells or portion of the plate to read
- **Default Protocol:** all newly created protocols and Quick Reads are based on the Default Protocol. If some protocol elements, like plate layout, runtime prompts, report headers and footers, etc., are largely the same for most of your projects, you'll save significant time by defining these elements before creating the next protocol/experiment
- **Print Preview:** save time and paper by viewing reports on-screen before sending them to the printer

Chapter 4

Assay Examples

This section contains step-by-step instructions for programming commonly known assays using Gen5. Gen5 also ships with Sample Protocols for most of these assays. Both the Sample Protocols and the assay descriptions are learning tools. It is your responsibility to customize and validate them to meet your needs.

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How do I set up my assay?	54

Sample Protocols and Experiments

Numerous sample protocols are shipped with Gen5. You can use the protocols to learn more about Gen5 and as a timesaver, customizing them to meet your needs and then running them in an experiment to obtain results.

❖ **Recommendation:** Before making any modifications to the sample protocols, we recommend selecting **File>Save As** after opening them and assigning a unique name to the protocol. This will preserve the original sample protocol for future use.

A matching experiment file is also shipped with Gen5 for use as a learning tool. Many of the experiment files contain actual data so you can see how Gen5 presents the results on-screen and in reports.



Find the sample protocols and experiments shipped with Gen5 in the default file storage locations. A folder for each detection method is available: Absorbance, Fluorescence, Luminescence and for Synergy 2 users, there is a Synergy 2 folder within each detection method folder:

- Gen5 Secure (and database users): Select **File>Open Protocol**, in the DB directory select the **Samples** folder.
- All other levels of Gen5: Select **File>Open Protocol** and browse to C:/Program Files/BioTek/Gen5/Samples
- Gen5's **Welcome** screen also offers the option to open a **Sample File**

❖ **Important:** The sample protocols must be considered as examples provided for demonstration and guidance purposes. If you plan to use these protocols or similar ones in a real application, *it is your responsibility to validate the protocol parameters*, including the report and export content (if applicable), before using them.

❖ **Notes:** Your system administrator can change the path and filenames described above. If you cannot find the Samples folder, contact your system administrator. Also note, your reader may not support all of the sample protocols provided. Review the descriptions in the Samples Protocol Listing to see if your reader is compatible with the defined steps.

Sample Protocols and Experiments Guide

You can review a complete description of samples in the Sample Protocols and Experiments Guide.PDF shipped with Gen5: click the Windows® **Start**, select **All Programs>Gen5>Sample Protocols and Experiments Guide**

❖ Gen5 installs a copy of the Sample Protocols and Experiments Guide in the Samples folder of the main Gen5 directory. (By default this is C:\Program Files\BioTek\Gen5\Samples)

You can also find a summary listing and brief description of the sample protocols in Gen5's Help. Review the description of the sample protocol to make sure it is compatible with your reader.

How do I set up my assay?

Here are step-by-step instructions for creating Gen5 Protocols to run common assays. (More Assay Examples can be found in Gen5's Help.) We hope that by following the instructions, making some changes to names and other details, you can adapt them for use in your lab. Also see the **Kinetic Analysis** chapter.

Absorbance

- Quantitative ELISA Example on page 55
- Subtracting Blank Plate Reads on page 58
- Pathlength Correction Example on page 60
- Dual Wavelength Absorbance Endpoint on page 62
- Basic Spectrum Analysis on page 64
- Protein Quantification Assay on page 66
- Max Binding Determination on page 83
- Toxicity/Cytotoxicity Assay on page 86
- Endotoxin Test on page 89
- β -Galactosidase Kinetic Assay on page 92

Fluorescence

- Basic Fluorescence Assay on page 69
- Kinetic Fluorescence Assay on page 71
- Fluorescence Assay with Injection on page 74
- Fluorescence Area Scan Example on page 77
- Fluorescence Polarization on page 79

Luminescence

- Basic Luminescence Glow Assay on page 81
- Luminescence Flash Assay with Injection on page 82

Dispensing Reagent

- Dispensing Reagent in a Kinetic Analysis on page 94
- Dispensing Reagent in an Endpoint Analysis on page 95

Quantitative ELISA Example

To help you set up your own assay here is an example of the steps required to run a quantitative ELISA assay. In this example we set up an endpoint Absorbance read, subtract Blank wells from all others, plot a standard curve, and define a Control to express the samples as a percentage of the control.

 It may be easier to follow these instructions if you have already watched the Gen5 Basic series of online tutorials: select Help>Tutorials or if you've completed the learning exercises described in the Getting Started Guide.

To set up the protocol, we'll define the:

1. Reading Procedure
2. Plate Layout
3. Data Reductions

❖ [Reporting Results](#) is the same process for all types of experiments

1. Defining the reading Procedure

This assay example has the simplest read Procedure: a single-wavelength Absorbance endpoint read:

1. Select **File>New Protocol**
2. Select **Protocol>Procedure**
3. Click the **Read** button and select the wavelength. Use the drop-down list or type the wavelength in the text field (overwrite the current value).
4. Click **OK** twice to save the Procedure

2. Defining the Plate Layout

This step is critical for the data reduction steps to be defined later. Here's the plate layout we need:

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	BLK	BLK	CTL1	CTL1	CTL1	SPL8	SPL8	SPL8	SPL16	SPL16	SPL16
B	STD1 0	STD1 0	STD1 0	SPL1	SPL1	SPL1	SPL9	SPL9	SPL9	SPL17	SPL17	SPL17
C	STD2 10	STD2 10	STD2 10	SPL2	SPL2	SPL2	SPL10	SPL10	SPL10	SPL18	SPL18	SPL18
D	STD3 20	STD3 20	STD3 20	SPL3	SPL3	SPL3	SPL11	SPL11	SPL11	SPL19	SPL19	SPL19
E	STD4 30	STD4 30	STD4 30	SPL4	SPL4	SPL4	SPL12	SPL12	SPL12	SPL20	SPL20	SPL20
F	STD5 40	STD5 40	STD5 40	SPL5	SPL5	SPL5	SPL13	SPL13	SPL13	SPL21	SPL21	SPL21
G	STD6 50	STD6 50	STD6 50	SPL6	SPL6	SPL6	SPL14	SPL14	SPL14	SPL22	SPL22	SPL22
H	STD7 60	STD7 60	STD7 60	SPL7	SPL7	SPL7	SPL15	SPL15	SPL15	SPL23	SPL23	SPL23

The critical factor is using the Well IDs, not their location on the plate. We did not need to customize the Well IDs for this example. We simply selected the Type, defined the known concentration of the standards and assigned them to the plate:

Well ID	Type	Description
BLK	Blank	DI water only
STD	Standard	Known concentrations
CTL1	Assay Control	Known Control
SPL	Sample	Unknown samples

Find specific instructions in the Preparing Plates chapter.

3. Defining the Data Reduction Steps

Now that we've defined the reading parameters and plate layout, we can define the data reduction steps: blank-well subtraction, standard curve, and expressing samples as a percentage of the control. Gen5 creates the blank-subtraction step for you automatically.

1. Select **Protocol > Data Reduction**
Notice that one Transformation, named "Blank nnn" where nnn is the wavelength, has already been created. We'll use the results of this calculation to plot the standard curve.
2. Click **Curve Analysis**

3. Notice on the **Data In** tab, the **Well ID** is set to STD and X Axis Data to <Plate Layout Settings>. The known concentrations entered for Standards are plotted on the X Axis. Use the drop-down list for the **Y Axis Data** to select Blank *nmn* (*wavelength*)
 4. Click the **Curve Fit** tab: depending on your assay, you may want to change the curve fit method to 4 Parameters or another option, or use Log values on the X or Y axis. For now, retain the defaults and click the **Data Out** tab. Take note that the **Data Set Name** produced from the standard curve is called Conc (by default. You can change it.). Click OK to save and close the curve.
 5. Click **Transformation**
 1. For the **Data In** use the drop-down list to select Conc.
 2. Enter a **New Data Set Name** for the results of this calculation, e.g. %Control
 3. In the **Formula** field enter: $(X/CTL1)*100$
Retain the default setting to Use single formula for all wells. X represents the value of the current well. CTL1 is the well ID for the control we assigned in Plate Layout.
-

 After the plate is read, you can return to the Data Reduction dialog to make any needed changes, like the Curve Fit Method. Do not change the Data Out or Data Set Names, this would invalidate the data reduction steps that use those data sets.

6. Save the protocol.

Now you're ready to define your reporting requirements, and run the protocol in an experiment.

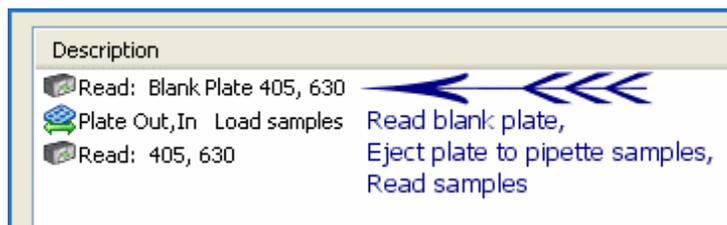
Subtracting Blank Plate Reads

To perform a blank-plate subtraction in your experiment, set up an additional **Read Step** for the blank plate, and then, create a **Data Reduction Transformation** to subtract the measurements of the blank plate from the samples plate.

You can insert a **Plate in/Out** step in the **Procedure** sequence to first read the blank plate, pause the experiment to pipette samples to the plate, and then, read the samples plate.

Step-by-step procedure:

1. Select **File>New Protocol**
2. Double click **Procedures** to set the reading parameters:
 - 1 First, create a **Read Step** for the blank plate: enter **Blank** for the Step Label to easily identify the raw data.



- 2 Add a **Plate In/Out** step to eject the plate to pipette samples, standards, etc. Optionally, enter "Load Samples" in the comment field.
- 3 Finally, create a **Read Step** for the samples plate.

❖ Other steps can be included in the sequence, like Set Temperature and Shake, if required.

3. Set up the **Plate Layout** to match the distribution of samples and standards or controls.
4. Double click **Data Reduction** to define a transformation: Blank Plate Data Reduction
 - 1 The dialog will contain any automatically generated data reductions. Highlight the top-most one and click **Transformation**, to position the blank subtraction as the first calculation.
 - 2 Click
 - 3 In the **Multiple Data Sets** screen, use the drop-down lists to select the **Blank** plate read data for **DS1** and the samples plate data for **DS2**.

❖ In multiple wavelength protocols there may be several data sets to choose from. If you've used the **Step Labels** for each Read Step, it's easy to match up the blank plate with the samples plate.

Data Sets	Plate	Data In
<input type="radio"/> DS1:	<Current Plate>	blank:A1(390)
<input checked="" type="radio"/> DS2:	Current Plate	test:A1(390)
<input type="radio"/> DS3:	Current Plate	blank:A1(390) blank:A2(490) blank:A3(630)
<input type="radio"/> DS4:	Current Plate	test:A1(390) test:A2(410) test:A3(630)

- 4 Enter a **New Data Set Name** for the resulting data set, e.g. Blanked 390
 - 5 Enter **DS2-DS1** in the **Plate Formula** field and click OK.
 - 6 Repeat steps 2-4 to create as many blank-plate subtraction Transformations as needed, e.g. one per wavelength.
 - 7 Now you're ready to create other Data Reduction steps using the blanked data sets. For example, select Curve Analysis to generate a standard curve based on the blank-subtracted test plate.
5. Customize the [Data Views](#) and fine-tune the [Report Builder](#) as needed before saving and closing this protocol. **File>Save**

Now you're ready to run the protocol in an experiment: **File> New Experiment**

Running the experiment:

After reading the blank plate, Gen5 ejects the carrier so you can load the samples



If you entered a comment, e.g. Load Samples in the Plate In/Out step, it is displayed on screen. Here's how to proceed:

- Click **OK after loading the samples**, when you're ready to continue reading the plate

Pathlength Correction Example

Here is an example of the steps required to perform pathlength correction in an ELISA assay. In this example we set up an endpoint Absorbance read, subtract Blank wells from all others, and transform the data to determine the concentrations of the unknown samples. This is the process used to create the Direct Oligo Quantification assay shipped as a sample protocol with Gen5.

 It may be easier to follow these instructions if you have already watched the Gen5 Basic series of online tutorials: select Help>Tutorials or if you've completed the learning exercises described in the Getting Started Guide.

To set up this protocol, we'll define the:

1. Reading Procedure
2. Plate Layout
3. Data Reductions

❖ **Reporting Results** is the same process for all types of experiments

1. Defining the reading Procedure

This assay example has the simplest read Procedure: a single-wavelength Absorbance endpoint read:

1. Select **File>New Protocol**
2. Select **Protocol>Procedure**
3. Click the **Read** button and keep the default settings for Detection Method, Read Type and Read Speed
4. Fill in the checkbox next to **Pathlength Correction**. [Optionally, click the 3-dot button to view (and modify if desired) the test and reference wavelengths used in the process.]
5. Set the **Wavelength**. Use the drop-down list or type the wavelength in the text field (overwrite the current value). For this example, enter 260.
6. Click **OK** twice to save the Read step and the Procedure

2. Defining the Plate Layout

For this assay example, the plate layout is very simple, comprising two blank wells and 94 unknown samples:

1.  Select **Protocol> Plate Layout**

2. In the **Well Settings** box, select the **Type** of specimen, first Blanks, then Samples
3. Assign the blanks to cells A1 and B1
4. Change the **Type** to Sample, make sure **Next ID** is enabled, click and drag over the remaining wells to assign the unknown samples

3. Defining the Data Reduction Steps

After defining the reading parameters and plate layout, we can define the data reduction steps. Gen5 creates the blank subtraction and the pathlength correction for you automatically.

1. Select **Protocol > Data Reduction**
Notice the two Transformations, "Blank 260" and "Corrected [Blank 260]", Gen5 first subtracts the blanks and then applies the Pathlength Correction Calculation.
2. Click **Transformation** to add another Data Reduction step
3. For the **Data In** use the drop-down list to select **Corrected [Blank 260]** data set
4. Enter a **New Data Set Name** for the results of this calculation, e.g. Concentration
5. In the **Plate Formula** field enter: **X*32.5**
Retain the default setting to Use single formula for all wells. X represents the value of the current well. The extinction coefficient for ssDNA oligonucleotides (1 mg/ml) at 260 nm is 13 ODs for a 1 cm pathlength; this can be recalculated to mean 1.0 OD has a concentration of 32.5 µg/ml.
6. Save the protocol.

Now you're ready to define your [reporting requirements](#), and run the protocol in an [experiment](#).

Dual Wavelength Absorbance Endpoint

Here are step-by-step instructions for setting up a dual-wavelength absorbance read with known concentrations of standards against which a linear regression curve is plotted.

Create the protocol:

1. Select **File>New Protocol**
2.  Double-click **Procedure** in the menu tree:
 - Click **Read** to set the reading parameters: Keep the default settings for Detection Method and Read Type: Absorbance Endpoint
 - For **Wavelengths**, click the button for **2** and use the drop-down list to select (or enter) the test and reference wavelengths: 410 and 630 for this example.
 - Click **OK** twice to close the Read Step, and then, the Procedure dialogs.
3.  Double-click **Plate Layout** to define the location of standards, samples, and blanks on the microplate. For this example, the standards are placed in the center of the plate, modify the instructions to match the distribution of samples and standards on your plate:
 - Set the Well Settings Type to **Standard** and click the 3-dot button next to the **Conc.** field to enter the expected concentrations. For this example, leave 0 in the STD1 cell at the top of the table. Select **Incr.** with a tick mark, and enter 10 in the field, then click in the **STD1** cell, then in the STD2 cell, and each subsequent cell in the table until STD8. Click **OK** to save and close the concentrations.
 - At the grid, set the Number of **Replicates** to 2, and select **Next Conc.** under **Auto Select**. Click and hold as you roll the mouse over the **5** and **6** columns, (the cursor changes to a black, down-facing arrow) to fill the entire columns.
 - Set the Well Settings Type to **Blank**, keep the Number of **Replicates** at 2, and click and drag over wells A1 and A2.
 - Set the Well Settings Type to **Sample**, keep the Number of **Replicates** at 2, and select **Next ID** under **Auto Select**. Click and drag the cursor over the remaining wells in columns 1 and 2, and then 3-4, and then 7-12, to assign samples to all the other wells of the plate.
4.  Double-click **Data Reduction**
Gen5 automatically creates the Blank-Subtraction transformations.
 - Click **Transformation** to set up the calculation:
5. Click

6. For **DS1** (selected by default) use the drop-down list to select **Blank 410**
7.  Select **DS2** and use the drop-down list to select **Blank 630**
8. Click **OK** to close the **Multiple Data Set** dialog
9. For this example, we'll call the **New Data Set Name: Dual Wavelength**. Enter the name in the text box. Dual wavelength is also known as **Delta OD**, you may want to use this name instead .
10. In the **Plate Formula** field enter: **DS1-DS2** to subtract the reference wavelength (630) measurements from the test (410) measurements. Retain the default: Use single formula for entire plate.
11. Click **OK** to save and close the **Transformation**
 - Click **Curve Analysis**. In the Data In tab, use the drop-down list to select **Y Data: Dual Wavelength** and click **OK**.

❖ For this simple protocol, the remaining default settings are acceptable. More options are available, like customizing the names of data sets, plotting interpolations in the generated curve, and so on. See [Plotting a Curve](#) in the Data Reduction Options chapter.

- Click **OK** to close the Data Reduction dialog.
12.  Set the **Report** parameters and **Data Views** as desired. For instructions, see [Viewing Results](#).
 13. Save the protocol: select **File>Save** and name it **DualWave1** for this example.

Run the protocol:

Now, you're ready to run the DualWave1 protocol in an experiment.

1. Select **File>New Experiment**. By default, Gen5 highlights the DualWave1 protocol in the dialog, making selection quick and easy.
2. If the reader is all set up, you're ready to go: Click **Read** and follow the online prompts.

Basic Spectrum Analysis

Numerous applications can profit by a preliminary spectral screening. Here are instructions for setting up a basic spectrum protocol in Gen5.

 It may be easier to follow these instructions if you have already watched the Gen5 Basic series of online tutorials: select Help>Tutorials, or if you've completed the learning exercises described in the Getting Started Guide.

1. Defining the reading Procedure

This assay example uses a kinetic read for analysis.

1. Select **File>New Protocol**
2. Open the **Procedure** (double click Procedure in the menu tree)
3. Click **Read** and change the **Read Type** to **Spectrum**
4. Set the Wavelength range: for this exercise set **Start** to **200** and **Stop** to **550**
5. Set the **Step** to **3**, and close the Read step
6. Click **OK** to save and close it.

2. Defining the Plate Layout

Define the plate layout in the usual way to reflect the arrangement of unknown samples, standards and blanks on the microplate.

3. Defining the Data Reduction Steps

Now that you've defined the reading parameters and plate layout, you can define the data reduction steps:

1. Select **Protocol> Data Reduction**
Gen5 automatically sets up the a Well Analysis for Min/Max OD. If Blanks have been assigned to the plate, it will be preceded by and based on a blank-subtraction Transformation step.
2. Click **Well Analysis** to add another step
3. Enter a unique name for this step in the **Label** field
4. Select one of the offered **Calculation Types**: Integral or Formula
5. Click **OK** to save and close the step
6. Click **OK** to save and close Data Reduction

4. Save the Protocol

1. Define the [Reporting Requirements](#) using the Report Builder or export options
2.  Save the protocol

Now you can run it in an experiment: select **File>New Experiment**

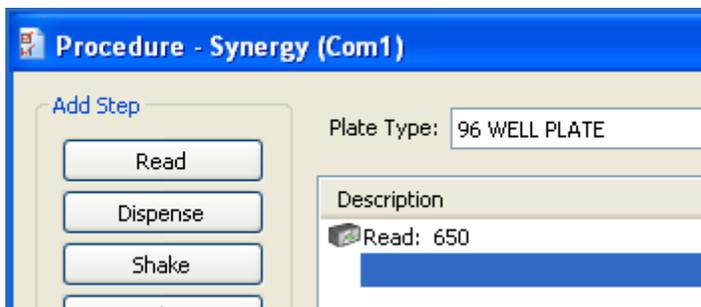
5. Viewing the Results

After you read the plate, you can take advantage of Gen5's [Well Zoom](#) to examine the results. This feature is described in the Kinetic Analysis chapter.

Protein Quantification: Endpoint Absorbance

Here are instructions for the Gen5 portion of running this type of assay — the easy part. Correctly mixing and dispensing the standards, and pipetting reagents to the plate is the tricky part. Follow the assay instructions closely and modify these steps, as needed. Click the links for instructions at each step.

1. Select **File>New Protocol**
2. Define the **Procedure**



1.  Select **Protocol>Procedure**
2. Click **Read** to add one read step
3. Keep the default settings:
 - Detection Method = **Absorbance**
 - Read Type = **Endpoint**
4. Set the **Wavelength** using the drop-down or enter **650** in the nm field
5. Click **OK** twice to close and save the **Read** step and the **Procedure**

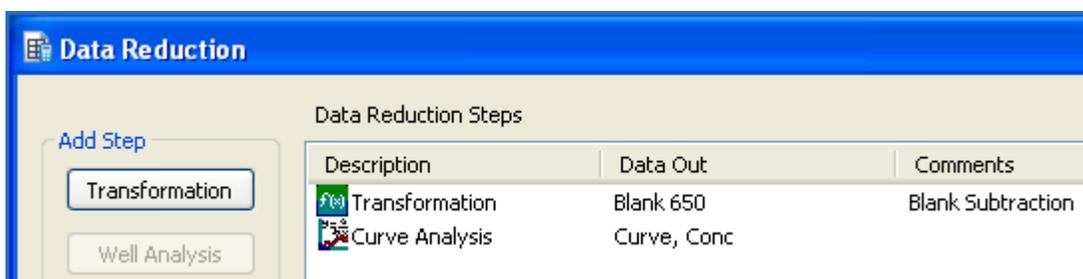
3. Define the Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD1 0	STD1 0	STD1 0	STD1 0	SPL1	SPL1	SPL1	SPL1	SPL9	SPL9	SPL9	SPL9
B	STD2 1	STD2 1	STD2 1	STD2 1	SPL2	SPL2	SPL2	SPL2	SPL10	SPL10	SPL10	SPL10
C	STD3 2.5	STD3 2.5	STD3 2.5	STD3 2.5	SPL3	SPL3	SPL3	SPL3	SPL11	SPL11	SPL11	SPL11
D	STD4 5	STD4 5	STD4 5	STD4 5	SPL4	SPL4	SPL4	SPL4	SPL12	SPL12	SPL12	SPL12
E	STD5 10	STD5 10	STD5 10	STD5 10	SPL5	SPL5	SPL5	SPL5	SPL13	SPL13	SPL13	SPL13
F	STD6 20	STD6 20	STD6 20	STD6 20	SPL6	SPL6	SPL6	SPL6	SPL14	SPL14	SPL14	SPL14
G	BLK	BLK	BLK	BLK	SPL7	SPL7	SPL7	SPL7	SPL15	SPL15	SPL15	SPL15
H					SPL8	SPL8	SPL8	SPL8	SPL16	SPL16	SPL16	SPL16

Set up Gen5's plate layout to match your placement of samples and standards on the plate, for example:

-  Select **Protocol > Plate Layout**
- In the Well Settings box, select the **Type** of specimen, first **Standards**, then **Blanks**, then **Samples**
-  Define the **Concentration** of the Standards:
- Set the **Replicates** to 4
- Assign the well IDs to their corresponding locations in the plate matrix by clicking in the respective wells in the matrix. Use the **Auto Select** options to speed up your work.

4. Define the Data Reduction:



Data Reduction Steps		
Description	Data Out	Comments
 Transformation	Blank 650	Blank Subtraction
 Curve Analysis	Curve, Conc	

Gen5 creates a Blank Subtraction data set when you put blanks on the plate, as defined above. Click in the white space below the Transformation step:

- Click **Curve Analysis** to create a standard curve:
- Data In: Well ID** is set to **STD**. Set the **Y Axis = Blank [650]**

- **Curve Fit:** Method is set to **Linear Regression**

5. Define the **Reporting Requirements**

Save the Protocol

Select **File > Save** when you're finished setting up the protocol. You'll be able to use this protocol repeatedly to run this assay in an experiment.

Select **File > New Experiment** and select the protocol when you're ready to run it, i.e. reagents are reconstituted, the plate is prepared, etc.

Basic Fluorescence Assay Example

To help you set up your own assay in Gen5 here is an example of the steps required for nucleic acids quantitation using a fluorescent stain, such as the dsDNA specific PicoGreen™.

1. Defining the reading Procedure

This assay example defines a single-filter-set Fluorescent endpoint read:

1. Select **File>New Protocol**
2. Select **Protocol>Procedure**
3. Click the **Read** button and change the **Detection Method to Fluorescence**
4. To set the **Filter Set**, use the drop-down list to select the filter for **Excitation** and **Emission**, for this exercise **485/20** and **528/20**, respectively
6. Keep the **Optics Position** set to **Top** for Synergy HT and FLx800; for Synergy 2 select a mirror that corresponds to the selected filters, e.g. **Top 510**. Top 50% works with any filter.
7. Enter **65** for the **Sensitivity** setting
8. Click **OK** twice to save and close the Procedure

2. Defining the Plate Layout

Define the plate layout in the usual way to reflect the arrangement of unknown samples, standards and blanks, if any, on the microplate. For the sample protocol shipped with Gen5, we set up the plate this way:

	1	2	3	4	5	6	7	8
A	STD1 0	STD1 0	SPL4	SPL4	SPL12	SPL12	SPL20	SPL20
B	STD2 25	STD2 25	SPL5	SPL5	SPL13	SPL13	SPL21	SPL21
C	STD3 250	STD3 250	SPL6	SPL6	SPL14	SPL14	SPL22	SPL22
D	STD4 2500	STD4 2500	SPL7	SPL7	SPL15	SPL15	SPL23	SPL23
E	STD5 25000	STD5 25000	SPL8	SPL8	SPL16	SPL16	SPL24	SPL24
F	SPL1	SPL1	SPL9	SPL9	SPL17	SPL17	SPL25	SPL25
G	SPL2	SPL2	SPL10	SPL10	SPL18	SPL18	SPL26	SPL26
H	SPL3	SPL3	SPL11	SPL11	SPL19	SPL19	SPL27	SPL27

Defining the expected concentration of the standards is the required to plot a curve. Find specific instructions in the Preparing Plates chapter.

3. Defining the Data Reduction Steps

With the reading parameters and plate layout defined, Data Reduction steps can be created: a standard curve for determining the concentrations of the unknown samples:

1. Select **Protocol> Data Reduction**
2. Click **Curve Analysis**
3. Notice on the **Data In** tab, the **Well ID** is set to **STD** and **X Axis Data** to <Plate Layout Settings>. The known concentrations entered for Standards are plotted on the X Axis. Use the drop-down list for the **Y Axis Data** to select **485/20,528/20**
4. Retain the default settings for **Curve Fit** and the **Data Out** tab. Take note that the **Data Set Name** produced from the standard curve is called **Conc** (by default. You can change it.). Click **OK** to save and close the curve.
5. Click **OK** twice to save and close Data Reduction

 After the plate is read, you can return to the Data Reduction dialog to make any needed changes, like the Curve Fit Method. Do not change the Data Out or Data Set Names, this would invalidate the data reduction steps that use those data sets.

4. Save the Protocol

1. Define the [Reporting Requirements](#) using the Report Builder or export options
2.  Save the protocol

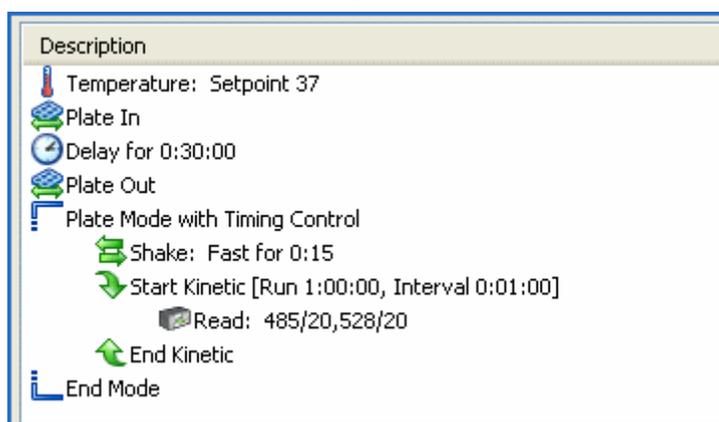
Now you can run it in an experiment: select **File>New Experiment**

Kinetic Fluorescence Assay Example

To help you set up your own assay in Gen5 here is an example of the steps required to measure antioxidant capacity based on the free radical damage to a fluorescent probe, as in the Oxygen Radical Absorbance Capacity (ORAC) Assays. This protocol deploys Gen5's **Synchronized Plate Mode** for precise timing of the measurements.

1. Defining the reading Procedure

This assay example defines a single-filter-set Fluorescent endpoint read:



1. Select **File>New Protocol**
2. Select **Protocol>Procedure**
3. Click **Set Temperature** and enter 37 for the temperature
4. Click **Plate Out/In** and select Move plate in
5. Click **Delay** and enter 0:30:00 minutes to let the reader warm up
6. Click **Plate Out/In** and select Move plate out
7. Under Synchronized Modes, click **Plate** for a synchronized-plate mode block
8. Click **Shake** and change the Intensity to Fast and the Duration to 0:15 seconds
9. Click **Kinetic** and set the Run Time to 1:00:00 hour and the Interval to 0:01:00 minute
10. Click the **Read** button and change the Detection Method to Fluorescence
11. To set the **Filter Set**, use the drop-down list to select the filter for **Excitation** and **Emission**, for this exercise **485/20** and **528/20**, respectively
12. Keep the **Optics Position** set to **Top** for Synergy HT and FLx800; for Synergy 2 select a mirror that corresponds to the selected filters, e.g. **Top 510**. Top 50% works with any filter.
13. Enter **65** for the **Sensitivity** setting
14. Click **OK** twice to close and save the Read step and the Procedure

2. Defining the Plate Layout

Define the plate layout in the usual way to reflect the arrangement of unknown samples, standards, controls and blanks on the microplate. For the sample protocol shipped with Gen5, we set up the plate this way:

	1	2	3	4	5	6	7	8	9	10	11
A											
B		STD6 100	STD4 25	STD2 6.25	SPL1	SPL3	SPL5	SPL7	SPL9	SPL11	BLK
C		STD6 100	STD4 25	STD2 6.25	SPL1	SPL3	SPL5	SPL7	SPL9	SPL11	BLK
D		STD6 100	STD4 25	STD2 6.25	SPL1	SPL3	SPL5	SPL7	SPL9	SPL11	BLK
E		STD5 50	STD3 12.5	STD1 0	SPL2	SPL4	SPL6	SPL8	SPL10	CTL1	BLK
F		STD5 50	STD3 12.5	STD1 0	SPL2	SPL4	SPL6	SPL8	SPL10	CTL1	BLK
G		STD5 50	STD3 12.5	STD1 0	SPL2	SPL4	SPL6	SPL8	SPL10	CTL1	BLK

Defining the expected concentration of the standards is required to plot a curve. Find instructions in the **Preparing Plates** chapter.

3. Defining the Data Reduction Steps

Now that we've defined the reading parameters and plate layout, we can define the data reduction steps: two well analysis steps and a standard curve.

1. Select **Protocol > Data Reduction**
Gen5 automatically creates two steps: the **Blank Subtraction** Transformation and Well Analysis for **Max V**
2. Click **Well Analysis** to add another step to perform a calculation on the results of individual read intervals:
 6. Enter a unique **Label** for this step, e.g. AUC
 7. Select **Formula** and enter it in the text field:
 $(R1/R1)+(R2/R1)+(R3/R1)+(R4/R1)+(R5/R1)+(R6/R1)+(R7/R1)+(R8/R1)+\dots+(R61/R1)$. This formula normalizes the AUC (area under the curve), the results are used to plot the standard curve to determine unknown concentrations
 8. Click **OK**
3. Click **Curve Analysis** to plot a standard curve:
 1. On the **Data In** tab, select the **Y-Axis Data**. In this example, choose the Well Analysis "AUC: Formula Result [Blank 485/20, 528/20]"

- 2 Click **OK** twice to save and close the Curve and the Data Reduction dialog

 After the plate is read, you can return to the Data Reduction dialog to make any needed changes, like the Curve Fit Method. Do not change the Data Out or Data Set Names, this would invalidate the data reduction steps that use those data sets.

4. Save the Protocol

1. Define the [Reporting Requirements](#) using the Report Builder or export options
2.  Save the protocol

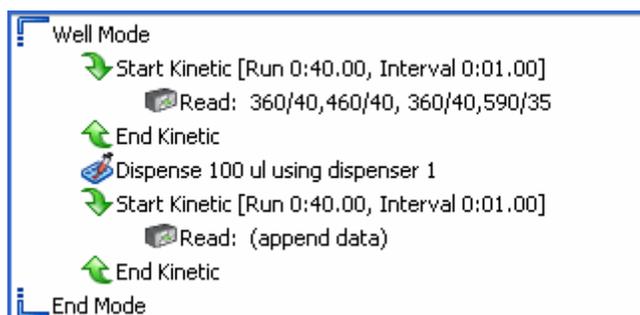
Now you can run it in an experiment: select **File>New Experiment**

Fluorescence Assay with Injection

To help you set up your own assay in Gen5 here is an example of the steps required to develop an ion channel assay and similar FRET assays which are well suited for sodium, potassium, calcium and ligand-gated ion channel research. This protocol deploys Gen5's Synchronized Well Mode to quickly switch between two emission filters in well kinetic mode.

1. Defining the reading Procedure

This assay defines a kinetic dual-filter-set Fluorescent read:



1. Select **File>New Protocol**
2. Select **Protocol>Procedure**
3. Under Synchronized Modes, click **Well** for a synchronized-well mode block
4. Click **Kinetic** and set the **Run Time** to 0:40.00 and the **Interval** to 00.15 second
5. Click the **Read** button and change the Detection Method to Fluorescence
 - 1 To set the **Filter Set**, use the drop-down list to select the filter for **Excitation** and **Emission**, for this exercise we set the first filter set to **360/40** and **460/40**, and the second filter set to **360/40** and **590/35**
 - 2 Set the **Optics Position** set to **Bottom** and enter **65** for the **Sensitivity** setting
 - 3 Click **OK** to close and save the Read step
6. In the Procedure workspace, highlight the **End Mode** step and click **Dispense**. Define the parameters for injecting the wells with **100 µl** of a High K⁺ solution to initiate depolarization. Select **Tip priming**
7. Click **Kinetic** and again set the **Run Time** to **0:40.00** and the **Interval** to **00.15** second
8. Click the **Read** button. Gen5 offers the limited-form read step because the parameters must match the first read step. De-select **Append to previous Kinetic data** and click **OK** (We're going to compare the before and after injection data sets, so we do not want them combined into one.)
9. Click **OK** to save and close the Procedure

2. Defining the Plate Layout

Define the plate layout in the usual way to reflect the arrangement of the cells and blanks on the microplate. For this assay we set it up the plate this way:

	1	2	3	4	5	6	7	8
A	BLK	SPL1	SPL9	SPL17	SPL25	SPL33	SPL41	SPL49
B	BLK	SPL2	SPL10	SPL18	SPL26	SPL34	SPL42	SPL50
C	BLK	SPL3	SPL11	SPL19	SPL27	SPL35	SPL43	SPL51
D	BLK	SPL4	SPL12	SPL20	SPL28	SPL36	SPL44	SPL52
E	BLK	SPL5	SPL13	SPL21	SPL29	SPL37	SPL45	SPL53
F	BLK	SPL6	SPL14	SPL22	SPL30	SPL38	SPL46	SPL54
G	BLK	SPL7	SPL15	SPL23	SPL31	SPL39	SPL47	SPL55
H	BLK	SPL8	SPL16	SPL24	SPL32	SPL40	SPL48	SPL56

Find instructions in the **Preparing Plates** chapter.

3. Defining the Data Reduction Steps

Now that we've defined the reading parameters and plate layout, we can define the data reduction steps. This type of FRET assay supports a direct comparison between the 590-nm and 460-nm fluorescence results via a ratiometric data reduction.

1. Select **Protocol > Data Reduction**
Gen5 automatically creates four **Blank Subtraction** Transformation steps and four Well Analysis for **Max V** (one for each data set)
2. Click **Transformation** to add another step to determine the ratio of the pre-injection (polarized) reads:
 - 1 Click the **Select multiple data sets** button: click the button for DS2, use the drop-down lists to select DS1: Blank Read 1:360/40,460/40 and for DS2: Blank Read 1: 360/40,590/35
 - 2 Enter a **New Data Set Name**, e.g. Polarized Em Ratio
 - 3 In the Plate **Formula** field enter DS1/DS2
 - 4 Click **OK**
3. Repeat Step 2 selecting Read 2 data sets to calculate the post-injection (depolarized) ratio: DS1: Blank Read 2:360/40,460/40 and for DS2: Blank Read 2: 360/40,590/35. For this assay we called the New Data Set: Depolarized Em Ratio
4. Create one more **Transformation** to calculate the response ratio:

- 1 Click the **Select multiple data sets** button: click the button for DS2, use the drop-down lists to select the previously calculated ratios, we named them DS1: Depolarized Em Ratio and for DS2: Polarized Em Ratio
 - 2 Enter a **New Data Set Name**, e.g. Response Ratio
 - 3 In the Plate **Formula** field enter DS1/DS2
 - 4 Click **OK**
 5. Perform additional Data Reduction steps, as needed, for further analysis outputs.
 6. Define the [Reporting Requirements](#) using the Report Builder or export options
 7.  Save the protocol
- Now you can run it in an experiment: select **File>New Experiment**

Fluorescence Area Scan Example

To help you set up your own assay in Gen5 here is an example of the steps required to perform a fluorescent area scan.

❖ The **Synergy 2**'s probe size limits its ability to perform Fluorescence area scan in plates with a small well diameter. Generally, this means you must use a plate with fewer than 96 wells.

1. Defining the reading Procedure

This assay example defines a single-filter-set Fluorescent area scan read on four wells:

1. Select **File>New Protocol**
2. Select **Protocol>Procedure**
 - Synergy 2 users must change the **Plate Type**
3. Click the **Read** button and change the **Detection Method** to **Fluorescence**
4. Set the **Read Type** to **Area Scan**
5. Click the **Full Plate** button in the upper right corner of the screen
6. Turn off the **Use all wells** by clicking the checkbox
7. Click and drag over the matrix to select four adjacent wells to read
8. Select the **Filter Set**
9. Keep the **Optics Position** set to **Top** for Synergy HT and FLx800; for Synergy 2 select a mirror that corresponds to the selected filters. Top 50% works with any filter.
10. Enter **65** for the **Sensitivity** setting
11. Click **OK** twice to save and close the Procedure

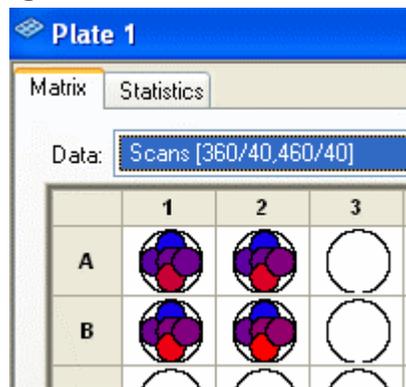
2. Defining the Plate Layout

Define the plate layout to reflect the arrangement of unknown samples, standards and blanks, if any, on the microplate. Find instructions in the **Preparing Plates** chapter.

3. Defining the Data Reduction Steps

Gen5 automatically creates a Well Analysis Data Reduction step to determine the Mean, Standard Deviation and CV% of the scanned wells. You can additional calculations as needed: **Protocol> Data Reduction**

4. Viewing the Results



To view the results on-screen:

1. Open the **Plate View**, and select the **Scans** data set in the **Data** field.
2. For a **Well Zoom** click on a well

Area scans can be used to determine the optimal settings for an assay.

Fluorescence Polarization Example

To help you set up your own assay in Gen5 here is an example of the steps required to perform a fluorescent polarization experiment.

 It may be easier to follow these instructions if you have already watched the Gen5 Basic series of online tutorials: select **Help>Tutorials** or if you've completed the learning exercises described earlier. Learn more about Fluorescence Polarization in Gen5's Help.

To set up the protocol, we'll define the:

1. Reading Procedure
2. Plate Layout
3. Data Reductions

❖ **Reporting Results** is the same process for all types of experiments

1. Defining the reading Procedure

This assay example defines a single-filter-set Fluorescent area scan read on four wells:

1. Select **File>New Protocol**
2. Select **Protocol>Procedure**
3. Click the **Read** button and change the **Detection Method** to **Fluorescence**
4. Select **Polarization** by clicking the checkbox
5. Optionally, you can change the default settings for **Light Source** and **Read Speed**
6. To set the **Filter Set**, use the drop-down list to select the filter for **Excitation** and **Emission**, for this exercise 485/20 and 528/20, respectively
7. Gen5 automatically sets the **Optics Position** to **Top** and selects the mirror in position 3. In the standard Synergy 2 configuration this is Top 510, which corresponds to the filters selected in the previous step. Since the mirror position is fixed for FP analysis, you must select a filter set that corresponds to the mirror in position 3.
8. Enter **65** for the **Sensitivity** setting
9. Click **OK** twice to save and close the Procedure

2. Defining the Plate Layout

Define the plate layout to reflect the arrangement of unknown samples, standards and blanks, if any, on the microplate.

3. Defining the Data Reduction Steps

Gen5 automatically creates the Fluorescence Polarization steps, preceded by the blank-subtraction transformations if Blanks were assigned to the plate layout. You can add other Data Reduction steps as needed: **Protocol > Data Reduction**

4. Save the Protocol

1. Define the reporting requirements using the Report Builder or export options
2.  Save the protocol.

Now you can run it in an experiment: select **File > New Experiment**

Basic Luminescence Glow Assay Example

To help you set up your own assay in Gen5 here is an example of the steps required to use reporter genes, such as luciferase, for studying gene expression. The step-by-step instructions provided here mirror the sample protocol shipped with Gen5 for Glow Luciferase Assay.

1. Defining the reading Procedure

This assay example defines a single-filter-set Luminescent endpoint read:

1. Select **File>New Protocol**
2. Select **Protocol>Procedure**
3. Click the **Read** button and change the **Detection Method** to **Luminescence**
4. Set the **Integration Time** to **1.0 SS.ss**
5. To set the **Filter Set**, use the drop-down list to select the **Hole** for **Emission**
6. Keep the **Optics Position** set to **Top** and enter **100** for the **Sensitivity** setting
7. Click **OK** twice to save and close the Procedure

2. Defining the Plate Layout

Define the plate layout in the usual way to reflect the arrangement of unknown samples, standards and blanks on the microplate. For the sample protocol shipped with Gen5, a Blank well and 47 unknown Sample wells in duplicate were defined.

Find instructions in the **Preparing Plates** chapter.

3. Defining the Data Reduction Steps

With the reading parameters and plate layout defined, Data Reduction steps can be created. Gen5 automatically creates the Blank Subtraction transformation. You can add additional calculations as needed.

4. Save the Protocol

1. Define the [Reporting Requirements](#) using the Report Builder or export options
2.  Save the protocol

Now you can run it in an experiment: select **File>New Experiment**

Luminescence Flash Assay with Injection

To help you set up your own assay in Gen5 here is an example of the steps required to use automated injection and luminescence detection for studying gene expression. The step-by-step instructions provided here mirror the sample protocol shipped with Gen5 for Flash Luciferase Assay.

1. Defining the reading Procedure

This assay example defines a single-filter-set Fluorescent endpoint read:

1. Select **File>New Protocol**
2. Select **Protocol>Procedure**
3. Click **Well** to set up a **Synchronized Well Mode** block
4. Click **Dispense** and set the **Volume** to **100** and the **Rate** to **300**
5. Click **Delay** to define a **Delay Time** of **0:02.00** (two seconds)
6. Click the **Read** button and change the **Detection Method** to **Luminescence**
 - 1 Set the **Integration Time** to 5.0 SS.ss
 - 2 To set the Filter Set, use the drop-down list to select the Hole for **Emission**
 - 3 Keep the **Optics Position** set to Top and enter 100 for the Sensitivity setting
 - 4 Click **OK** twice to save and close the Procedure

2. Defining the Plate Layout

Define the plate layout in the usual way to reflect the arrangement of unknown samples, standards and blanks on the microplate. For the sample protocol shipped with Gen5, 48 unknown Sample wells in duplicate were defined.

3. Defining the Data Reduction Steps

With the reading parameters and plate layout defined, Data Reduction steps can be created. Gen5 automatically creates the Blank Subtraction transformation. You can add additional calculations as needed.

4. Save the Protocol

1. Define the [Reporting Requirements](#) using the Report Builder or export options
2.  Save the protocol

Now you can run it in an experiment: select **File>New Experiment**

Max Binding Determination/Competitive Assay

To help you set up your own assay in Gen5 here is an example of the steps required to run a competitive ELISA assay to determine maximum binding. In this example we set up an endpoint Absorbance read, subtract Blank wells from all others, subtract NSB (non-specific binding) wells from all others, plot a standard curve, and define B/B₀ as a percentage of bound sample and identify the Total Activity (TA) wells.

1. Defining the reading Procedure

This assay example has a simple read Procedure: a single-wavelength Absorbance endpoint read:

1. Select **File>New Protocol**
2. Select **Protocol>Procedure**
3. Click the **Read** button and select the wavelength. Use the drop-down list or type the wavelength in the text field (overwrite the current value).
4. Click **OK** twice to save the Procedure

2. Defining the Plate Layout

This step is critical for the data reduction steps to be defined later. Here's the plate layout we need:

	1	2	3	4	5	6
A	BLK	BLK	SPL1	SPL1	SPL9	SPL9
B	NSB	NSB	SPL2	SPL2	SPL10	SPL10
C	B ₀	B ₀	SPL3	SPL3	SPL11	SPL11
D	TA	TA	SPL4	SPL4	SPL12	SPL12
E	STD1 100	STD1 100	SPL5	SPL5	SPL13	SPL13
F	STD2 50	STD2 50	SPL6	SPL6	SPL14	SPL14
G	STD3 5	STD3 5	SPL7	SPL7	SPL15	SPL15
H	STD4 0.5	STD4 0.5	SPL8	SPL8	SPL16	SPL16

The critical factor is using the Well IDs, not their location on the plate. We customized the Well IDs for this example, changing the first three Assay Control IDs to NSB, B₀, and TA. Then, defined the known concentration of the standards and assigned all of them to the plate:

Well ID	Type	Description
BLK	Blank	DI water only
NSB	Assay Control	Assay-specific
Bo	Assay Control	Assay-specific
TA	Assay Control	Assay-specific
STD	Standard	Known concentrations
SPL	Sample	Unknown samples

3. Defining the Data Reduction Steps

Now that we've defined the reading parameters and plate layout, we can define the data reduction steps: blank-well subtraction, NSB subtraction, determine the [percentage bound](#) and plot a standard curve. Gen5 creates the blank-subtraction step for you automatically.

1. Select **Protocol > Data Reduction**
 - Notice that one Transformation, named "Blank *nnn*" where *nnn* is the wavelength, has already been created. We'll use the results of this calculation to build the next step.
2. Click **Transformation**
 - 1 For the **Data In** use the drop-down list to select **Blank *nnn***.
 - 2 Enter a **New Data Set Name** for the results of this calculation, e.g. **B/Bo**
 - 3 In the **Formula** field enter: $(X-NSB)/(Bo-NSB)*100$
3. Click **Curve Analysis**
 - 1 Notice on the **Data In** tab, the **Well ID** is set to STD and **X Axis Data** to <Plate Layout Settings>. The known concentrations entered for Standards are plotted on the X Axis. Use the drop-down list for the **Y Axis Data** to select B/Bo (or whatever you named the New Data Set Name in the previous step.)
 - 2 Click the **Curve Fit** tab: depending on your assay, you may want to change the curve fit method to 4 Parameters or another option, or use Log values on the X or Y axis. For now, retain the defaults and click the Data Out tab. Take note that the Data Set Name produced from the standard curve is called Conc (by default. You can change it.).
 - 3 Click **OK** to save and close the curve.
4. Save the protocol.

Now you're ready to define your reporting requirements, and run the protocol in an experiment.

More Advanced Options

- **Monitor Wells:** Sometimes it is necessary/desirable to wait until a certain amount of activity has occurred in the plate before reading it. Gen5 will periodically check designated wells until they've reached a certain measurement when the Monitor Wells option is used. When the criterion is met, Gen5 continues the Procedure, i.e. the regular plate reading is performed.

Toxicity/Cytotoxicity Assay

To help you set up your own assay in Gen5 here is an example of the steps required to run a Toxicity or Cytotoxicity assay to determine LD50 (lethal dose). In this example the Read step is straightforward, but the Plate Layout takes full advantage of Gen5's ability to customize Well IDs. We'll create two samples with six dilutions each, and a control and blank for each sample. Then, we'll plot a standard curve based on blank-subtraction and toxicity percentage. We'll use Gen5's Curve Interpolation to identify LD50.

1. Defining the reading Procedure

This assay example has a simple read Procedure: a single-wavelength Absorbance endpoint read:

1. Select **File>New Protocol**
2. Select **Protocol>Procedure**
3. Click the **Read** button and select the wavelength. Use the drop-down list or type the wavelength in the text field (overwrite the current value). Alternatively, you may want to perform a kinetic analysis.
4. Click **OK** twice to save the Procedure

2. Defining the Plate Layout

This step is critical for the data reduction steps to be defined later. Here's the plate layout we need:

	1	2	3	4	5	6	7
A	SPL1:1 10	SPL1:1 10	SPL1:1 10		SPL2:1 10	SPL2:1 10	SPL2:1 10
B	SPL1:2 20	SPL1:2 20	SPL1:2 20		SPL2:2 20	SPL2:2 20	SPL2:2 20
C	SPL1:3 30	SPL1:3 30	SPL1:3 30		SPL2:3 30	SPL2:3 30	SPL2:3 30
D	SPL1:4 40	SPL1:4 40	SPL1:4 40		SPL2:4 40	SPL2:4 40	SPL2:4 40
E	SPL1:5 50	SPL1:5 50	SPL1:5 50		SPL2:5 50	SPL2:5 50	SPL2:5 50
F	SPL1:6 60	SPL1:6 60	SPL1:6 60		SPL2:6 60	SPL2:6 60	SPL2:6 60
G	CTL1	CTL1	CTL1		CTL2	CTL2	CTL2
H	BLK1	BLK1	BLK1		BLK2	BLK2	BLK2

The critical factor is setting up the Well IDs and dilution values. For the Samples, (notice there are only two samples on the plate) we defined the known dilution values and assigned them to the plate using the **Auto Select** features Next ID and Next Dil for 3 Replicates. We customized the Well IDs for the **Sample Control IDs** to BLK to have sample-specific blanks and assigned them to plate without using the Auto Select features. Assay Controls were assigned to the plate in the same way as the BLK1 and BLK2.

Well ID	Type	Description
SPL	Sample	Unknown samples with dilution values
CTL	Assay Control	Sample-specific
BLK	Sample Control	Customized to be sample specific

3. Defining the Data Reduction Steps

Now that we've defined the reading parameters and plate layout, we can define the data reduction steps: blank-well subtraction, toxicity percentage determination and a standard curve.

1. Select **Protocol > Data Reduction**
2. Click **Transformation**
 - 1 For the **Data In** only one data set, the raw data from the Read step is available.
 - 2 Enter a **New Data Set Name** for the results of this calculation, e.g. %Toxicity
 - 3 De-select the **Use single formula for all wells**
 - 4 In the **Current Formula** field enter: $(X-BLK1)/(CTL1-BLK1)*100$
 - 5 Click and drag over the **SPL1** wells to assign the formula to them
 - 6 Change the **Current Formula** for SPL2 wells: $(X-BLK2)/(CTL2-BLK2)*100$ and assign the formula to them
 - 7 Click **OK** to save and close the Transformation
3. Click **Curve Analysis**
 - 1 On the **Data In** tab, the **Well ID** is set to **SPL- All IDs** and **X Axis Data** to <Plate Layout Settings>. The known dilutions entered for Samples are plotted on the X Axis. Use the drop-down list for the **Y Axis Data** to select **% Toxicity** (or whatever you named the **New Data Set Name** in the previous step.)
 - 2 Click the **Curve Fit** tab and change the curve fit method to **4 Parameters** (unless you prefer another method).

- 3 Click the **Data Out** tab, in the **Interpolations** table enter **50** (and 90, if desired). 50 represents 50% toxicity and it will be plotted on the curve. Click **OK** to save and close the curve.
4. Save the Data Reduction steps (click OK)
5.  Save the protocol

Now you're ready to define your reporting requirements, and run the protocol in an experiment.

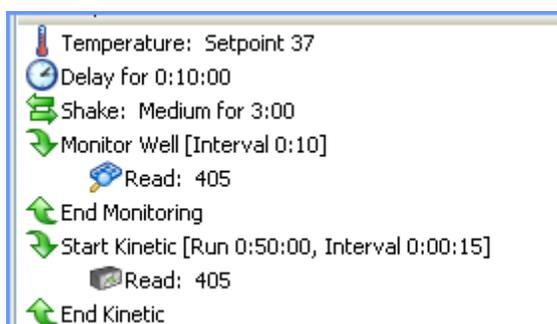
Endotoxin Test

Here are some **guidelines** for setting up a protocol to detect endotoxins in test samples using a Limulus Amebocyte Lysate assay kit.

- ❖ There are numerous variables when running this type of experiment. This example uses a kinetic analysis rather than an endpoint, for instance. **Modify these procedures to comply with your assay kit instructions.**

First, create a new Protocol: **Select File>New Protocol**

Set up the Procedure



1. Begin with **Set Temperature**
2. Add a **Delay** step to incubate the plate
3. Add a **Shake** step to mix the ingredients in the well
4. Add a **Monitor Well** step to delay the start time for collecting actual measurements until selected wells reach an OD of 0.05. Gen5 sets up well monitoring as a loop in the Procedure.
 - 1 Click **Monitor Well** to add the loop to the Procedure.
 - 2 Define the criteria that must be met before the reader moves onto the next step
 - 3 With the End Monitoring step highlighted, click **Read** to define the reading parameters for well monitoring.
 - 4 Select the wells to monitor and define the same reading parameters as the actual read step: Absorbance read at 405 nm.
5. Add **Kinetic** and set the **Run Time** to **50** minutes and the **Interval** to **15** seconds
6. Set the **Read** step to **Absorbance** at **405** nm.
7. Click **OK** twice to save and close the Procedure.

Define the Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	
A		Standards										
B		STD1 1	STD2 0.5	STD3 0.25	STD4 0.125	STD5 0.0625	STD6 0.03125		BLK			
C		STD1 1	STD2 0.5	STD3 0.25	STD4 0.125	STD5 0.0625	STD6 0.03125		BLK	Assay Controls		
D	Sample	SPL1:1 2	SPL1:2 4	SPL1:3 8	SPL1:4 16	SPL1:5 32	SPL1:6 64	SPL1:7 128		Pos		
E	w/ dilutions	SPL1:1 2	SPL1:2 4	SPL1:3 8	SPL1:4 16	SPL1:5 32	SPL1:6 64	SPL1:7 128		Pos		
F	Spiked	Spike1 1.25	Spike2 1.25	Spike3 1.25	Spike4 1.25	Spike5 1.25	Spike6 1.25	Spike7 1.25		Neg		
G	samples	Spike1 1.25	Spike2 1.25	Spike3 1.25	Spike4 1.25	Spike5 1.25	Spike6 1.25	Spike7 1.25		Neg		
H												

Take note of some of the conditions particular to this type of assay: Standards with decreasing concentration values, and only one Sample, with known dilution values, is assigned to the plate using the **Auto Select** features Next ID and Next Dil, in duplicate. For the "Spiked Samples" we customized the Well ID of Sample Controls (SPLC), changing it to Spike and assigned a concentration value. The Assay Control IDs were customized to Pos and Neg.

Well Type	ID
Standard	STD
Sample	SPL
Sample Control	Spike
Assay Control	Pos & Neg
Blanks	BLK

Set up Data Reduction

Data reduction, in this endotoxin example, requires a blank-subtraction, an onset OD well analysis, a standard curve and a transformation for % Recovery determination. Gen5 creates the blank-subtraction data set and a Max V Well Analysis step automatically.

1. Select **Protocol>Data Reduction**
Gen5 created the Blank 405 data set (if you defined this wavelength for the Read step) and used Blank 405 as the Data In for the Well Analysis step. (You can retain or replace the Max V step.)

2. Click **Well Analysis** to determine the **Onset OD** (if this is the second Well Analysis step, give it an unique Label):
 - 1 Set the **Data In** to **Blank 405**
 - 2 Select the button for **Onset OD**
 - 3 Enter **0.03** for the value.
3. Click **Curve Analysis** to create a standard curve:
 - 1 On the **Data In** tab: set the **Well ID** to **STD** and the **Y Axis Data** to **t at Total OD [405]**
 - 2 On the **Curve Fit** tab: select **Linear Regression** for the method and change the **X Axis Data** and **Y Axis Data Transformation** to **Log**
 - 3 On the **Data Out** tab: select both Calculate Concentrations options. Enter a **Data Set Name** for the **Concentration X Dilution** results: **Conc X Dil.**
2. Click **Transformation** to calculate the percentage recovery:
 - 1 For the **Data In** select **Conc X Dil**
 - 2 Enter a New **Data Set Name**: **% Recovery**
 - 3 De-select the **Use single formula for all wells**
 - 4 In the **Current Formula** field enter: **(Spike1-SPL1:1)/(.125)*100**
 - 5 Click in the **Spike1** wells to assign the formula to them
 - 6 Change the **Current Formula** for **SPL1:2** wells: **(Spike2-SPL1:2)/(.125)*100** and assign the formula to the Spike2 wells
 - 7 Change the **Current Formula** for **SPL1:3** wells: **(Spike3-SPL1:3)/(.125)*100** and assign the formula to the Spike3 wells
3. Repeat the process for all the spiked samples
4. Create two **Validation** steps:
 - 1 Click **Validation** and set the **Data In** = **t at Total OD [Blank 405]**
 - ◆ enter the **Formula** = **Neg>STD5*1.1**
 - 2 Click **Validation** and set the **Data In** = **% Recovery**
 - ◆ enter the **Formula** = **50%<Spike#<200%**

Set up the Report

Define your reporting requirements as with all other types of experiments.

Save the Protocol

Select **File>Save** when you're finished setting up the protocol. You'll be able to use this protocol repeatedly to run this endotoxin assay in an experiment. Select **File>New Experiment** and select the endotoxin protocol when you're ready to run it, i.e. reagents are reconstituted, the plate is prepared, etc.

β-Galactosidase

Quantitation of β-galactosidase (β-gal) enzymatic activity is a commonly used determination in cellular and molecular biology. A colorimetric assay using o-nitrophenol-B-D-galactoside (ONPG) as the substrate for β-galactosidase is described in an Application Note on BioTek's website. Here are instructions for setting up this type of assay in Gen5.

1. Defining the reading Procedure

This assay example uses a kinetic read for analysis.

1. Select **File>New Protocol**
2. Open the **StepWise Procedure**
3. Click **Kinetic**. Gen5 opens the Kinetic Step controls. Define the timelines:
 - Enter **0:30:00** minutes for the **Run Time**
 - Set the **Interval** to **0:00:30** seconds
4. With the **End Kinetic** step highlighted, click **Read** and set the Wavelength to **420**
5. At the **Procedure** dialog, click **OK** to save and close it.

2. Defining the Plate Layout

This assay requires 12 standards are decreasing concentration. The plate layout is critical to the data reduction steps defined later:

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD1 10	STD1 10	STD1 10	STD1 10	STD5 0.625	STD5 0.625	STD5 0.625	STD5 0.625	STD9 0.03907	STD9 0.03907	STD9 0.03907	STD9 0.03907
B	STD2 5	STD2 5	STD2 5	STD2 5	STD6 0.3125	STD6 0.3125	STD6 0.3125	STD6 0.3125	STD10 0.01954	STD10 0.01954	STD10 0.01954	STD10 0.01954
C	STD3 2.5	STD3 2.5	STD3 2.5	STD3 2.5	STD7 0.1563	STD7 0.1563	STD7 0.1563	STD7 0.1563	STD11 0.00977	STD11 0.00977	STD11 0.00977	STD11 0.00977
D	STD4 1.25	STD4 1.25	STD4 1.25	STD4 1.25	STD8 0.07815	STD8 0.07815	STD8 0.07815	STD8 0.07815	STD12 0	STD12 0	STD12 0	STD12 0
E	SPL1	SPL1	SPL1	SPL1	SPL5	SPL5	SPL5	SPL5	SPL9	SPL9	SPL9	SPL9
F	SPL2	SPL2	SPL2	SPL2	SPL6	SPL6	SPL6	SPL6	SPL10	SPL10	SPL10	SPL10
G	SPL3	SPL3	SPL3	SPL3	SPL7	SPL7	SPL7	SPL7	SPL11	SPL11	SPL11	SPL11
H	SPL4	SPL4	SPL4	SPL4	SPL8	SPL8	SPL8	SPL8	SPL12	SPL12	SPL12	SPL12

The Standard concentrations can be defined in Gen5 using the Auto entry tools:

STD1	10
STD2	5
STD3	2.5
STD4	1.25
STD5	0.625
STD6	0.3125
STD7	0.1563
STD8	0.07815
STD9	0.03907
STD10	0.01954
STD11	0.00977
STD12	0

1. Set the **Well Type** to **Standard** and click the 3-dot button next to the **Conc.** field
2. Enter **10** in the table for **STD1**
3. Fill the checkbox next to **Fact.** and enter **.5** in the text field
4. Click in the **STD2** cell in the table to apply the factor
5. Click in or use the down arrow key to move to each next cell until **STD12**
6. Change **STD12** to 0

Assign the location of the standards and unknown samples to the plate.

3. Defining the Data Reduction Steps

Now that we've defined the reading parameters and plate layout, we can define the data reduction steps: two well analysis steps and a standard curve.

1. Select **Protocol > Data Reduction**
Gen5 automatically sets up the first Well Analysis for MaxV
2. Click **Well Analysis** to add another step
 1. Enter a unique name for this step in the **Label** field, e.g. Mv for this exercise
 2. Select the **Mean V** Calculation Type
 3. Click OK to save and close the step
3. Click **Curve Analysis** to define a standard curve
 1. On the **Data In** tab set the Y-Axis Data to **Mv: Mean V[420]**
 2. On the Curve Fit tab select 4 Parameters
 3. Click OK to save and close the curve step
4. Click **OK** to save and close Data Reduction

4. Save the Protocol

1. Define the reporting requirements using the Report Builder or export options
2.  Save the protocol

Now you can run it in an experiment: select **File > New Experiment**

Dispensing Reagent

Dispensing reagent during an experiment is affected by the type of analysis. Select the option that most closely fits your requirements:

- [Dispensing Reagent in a Kinetic Analysis](#)
- [Dispensing Reagent in an Endpoint \(non-kinetic\) Analysis](#)

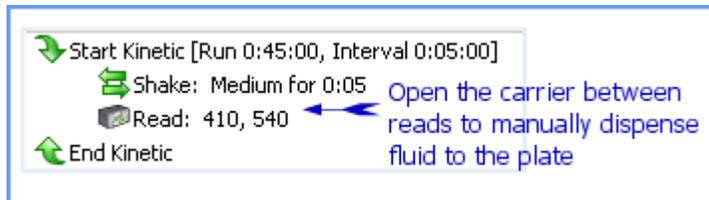
Dispensing Reagent in Kinetic Analysis Protocols

If dispensing is required during a kinetic read, here are two options:

Manually Dispensing Reagent:

Create a protocol with an extended kinetic interval; add enough time to dispense reagent to the plate between readings. During an interval, when the reader is idle:

1. Push the **carrier eject button** on the front of the reader to eject the plate
2. Perform the dispense using a pipette
3. Push the carrier eject button to draw the plate back in
4. The read will continue as scheduled



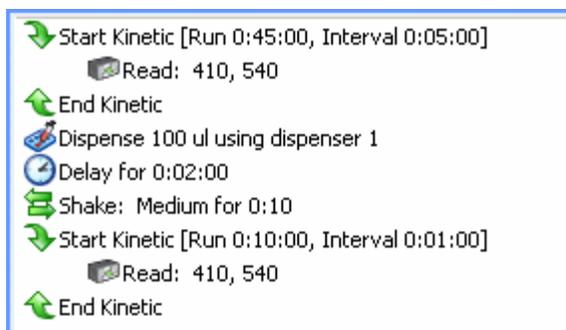
Readers with Injectors Dispensing:

(Synergy HT, Synergy 2, and FLx800 with Injectors)

Standard Mode:

Define two kinetic read steps around a dispense step in the **Procedures**:

1. Set the **Kinetic** timelines, define the **Reading** parameters, and **End** the loop.
2. After the loop, add a **Dispense** step to dispense the reagent. (You can add a Delay and/or Shake step after dispensing and before the next kinetic loop.)
3. Add a second **Kinetic** loop, kinetic settings can differ. Add a Read step to the loop, and check **Append to Previous Kinetic Data**. Selecting this option copies the previous defined reading parameters, e.g. wavelength/filter set, to this step.



Synchronized Mode:

For Fluorescence or Luminescence analysis select **Plate** or **Well** Mode to most precisely control the timing of your experiment. Learn [About Synchronized Modes](#) in Gen5's Help.

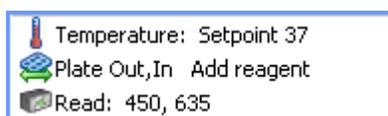
1. Add a Plate or Well mode block to the Procedure
2. Create a **Dispense** step to dispense the reagent. (You can add a Delay, and in Plate mode a Shake step, after dispensing and before reading.)
3. Add a Read step to the block, defining the reading parameters as needed.
4. Within the block you can repeat any or all of the options: Dispense, Delay, Shake in Plate mode, and Read, again. Note: the first Read step in the block sets the parameters

Dispensing Reagent in Endpoint Analysis

Manually Dispensing Reagent:

Add a Plate In/Out step to the Procedure:

1. You might want to incubate the plate before adding the reagent. If so, and your reader is capable, add a **Set Temperature** step.
2. Add a **Plate In/Out** step. Enter "Add Reagent" in the **Comment** field.
3. Add a Read Step.

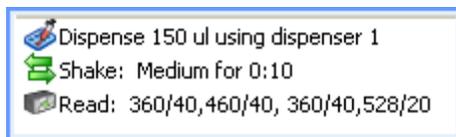


Readers with Injectors Dispensing:

(Synergy HT, Synergy 2, and FLx800 with Injectors)

Define a dispense step in the **Procedure**:

1. Create a **Dispense** step to dispense the reagent.
2. Add a Delay and/or Shake step after dispensing, if desired.
3. Add a **Read** step.



i Other Options

Multi-Detection Methods

For readers capable of performing multiple detection methods, like BioTek's Synergy, Gen5 supports multi-detection kinetic protocols.

Discontinuous Kinetic Procedure

A Discontinuous Kinetic Procedure can be defined to execute a sequence of readings over an extended time period. Use the Procedure's [Advanced Options](#) to conduct an experiment that requires long periods of downtime (for rest or incubation) between reads. Learn more in the Gen5 Help.

Chapter 5

Basic Tasks

This section provides instructions for performing basic tasks in Gen5. It also describes the process for creating an Experiment (based on a protocol).

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Quick Read

In Gen5, a Quick Read is using the microplate reader connected to the PC to read a plate and report the results. It's called quick because it is accomplished without taking the time to set up a protocol.

To perform a Quick Read:

1. Click **Read a Plate** from the Welcome page (and skip down to Step 4)
or Click  or select **File>New Experiment**
2. Select Default Protocol
3. Click  (the **Read** button)
The Procedures dialog opens.
4. Click  and enter the desired reading parameters, and any other needed steps. When you click **OK** to save and close the Procedure, the **Plate Reading** dialog opens.
5. Click **Read**

 When the reading is done you can report the results or select a view and click the **Quick Export** button to use Excel® to manipulate the data.

How to Create a Standard Curve

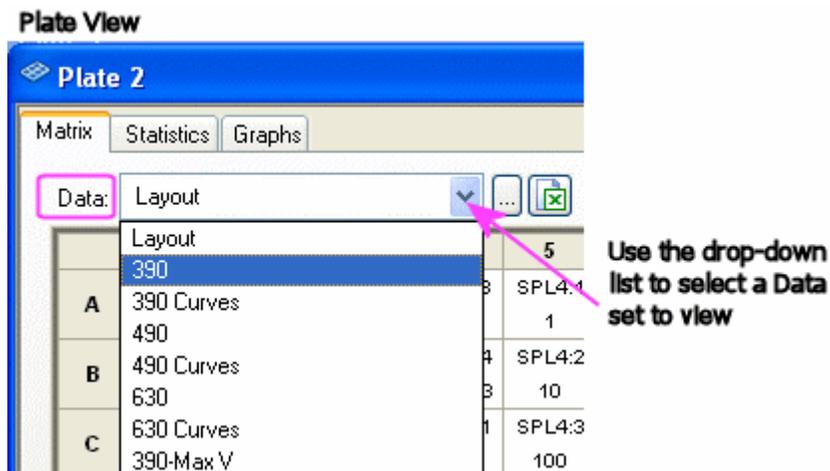
Gen5 lets you create one or more standard or calibration curves for determining the concentration of test samples:

1. Select **File> New Protocol**
2. Select **Procedure** and define the **Read** step (and any other required steps)
3. Select **Plate Layout**:
 - Define the **Concentrations of the Standards**
 - Assign the location of the standards, samples, and blanks (if any) on the plate
4. Select **Data Reduction> Curve Analysis**
 - Gen5 may have generated a "corrected" data set: if you assigned blanks to the plate or selected Pathlength Correction in the Read step, you'll want to select these data sets for **Data In** for the **Y-Axis Data** when plotting the curve
5.  On the Data In tab, use the drop-down to select the **Y-Axis Data**
6. On the Curve Fit tab, choose a curve fit method
7. Other options and requirements when defining multiple curves:
 - **Curve Name**: replace the default "Curve" with a more meaningful or unique name
 - On the Data Out tab, replace the default "Conc" for the **Data Set Name** with a more meaningful or unique name
 - On the Data Out tab, define interpolations to plot on the curve
8. Define the reporting or export requirements and **Save** the protocol. Now, you're ready to run an experiment: select **File> New Experiment** to read the plate and generate the curve.

Viewing Results

❖ Learn more in the **Viewing Results** chapter.

You can instantly view the results of an experiment in Gen5's main workspace using the **Plate View**:



- After reading the plate (or otherwise acquiring data), in the **Plate View** use the drop-down list for **Data** to display the raw data and any data reduction results
-  Click the **3-dot button** next to a data set to customize the view's appearance, including changing the numeric Format, e.g. number of decimal places, and the Font. (This feature is also available in the [Data Views](#) dialog.)
- **** Asterisks** are used to signal a change: in Gen5's title bar an asterisk indicates the current file has been changed but not-yet saved. When asterisks enclose a data set it has been become invalid. Generally this is because a Read step or Data Reduction step has been altered. Edit custom-made data views to select valid data sets
-  **384- and 1536-well plates** require resizing to effectively see the data. Gen5 adds a button to the **Plate View** to zoom in on the top-left quadrant of the plate and zoom out to view the entire plate. After zooming in, use the scroll bars to bring the other quadrants into focus. Find more on **resizing** the views in Gen5's Help
-  Click the **Quick Export** button to instantly open the current view in Excel®. Learn more about Gen5's Export Options



- [Multi-index readings](#) offer another viewing option. Kinetic and scanning reads generate views based on the number of read intervals, wavelengths, or

positions defined. Use the **spin** buttons or enter the desired read index and click **Show** to display it. Gen5 displays the time, wavelength, or position of the selected read number.

-  **Kinetic and Scanning protocols** can generate **Well Analysis** data sets labeled **Curves** in the Matrix drop-down list, open the Curves data set and click on a well for a [Well Zoom](#)

 Starting at the **Curves** data set, you can display multiple well zooms simultaneously by holding down the **Ctrl** key while selecting (up to 8) wells

- You can also select **Create new Matrix** to define a new view
- Select the **Statistics** tab to view a table of data reduction results
- Select the **Graphs** tab (when available) to view any Curves, except kinetic analysis curves, which Gen5 calls Well Analysis and is described above
- Select the **Cutoffs** tab (when available) to view the values or results of the cutoff formulas
- Select the **Validation** tab (when available) to view the values and results of the validation formulas
- Review this description of Gen5's naming convention for the raw data/results: **Data Set Naming** on page 306

Important Notes:

- Gen5 may not display some data points by default; to see them you must create your own [Data Views](#). If you expected to see certain results that are not currently displayed, try creating your own views.
- All data views are also available for Reporting and/or Exporting
- Gen5 always uses your computer's **Regional Settings** to display and input data.
- Modify a data view to change the way results are reported, including the number of decimal places and significant digits. Learn more in the **Viewing Results** chapter, including the meaning of the [Symbols and Notations](#) displayed

Printing Results

Gen5 offers numerous options for results output. Its report engine offers two primary outputs:

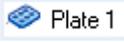
-  Click the **Print** button to print the results of an experiment **AFTER** you have created a report.
- **QuickPrint** instantly generates a print out of the current view or selection. After selecting the current view or specific content, right-click and select QuickPrint. Click and drag to select contiguous cells or hold down the Ctrl key and click to select non-contiguous cells.

Create a Report:



Before you can print a report, you must select the report content using Gen5's **Report Builder**.

Reporting in an experiment is done on a per plate basis:

- Highlight a  in the menu tree and select **Print/Print Preview**.
- **In a multi-plate experiment:** You can select multiple plates by holding the **Ctrl** key while highlighting them, or to select contiguously-ordered plates, highlight the first plate, hold down the **Shift** key and select the last plate. Then, click the **Print** button.



Gen5 offers enormous flexibility in report output. After defining the report elements, use the **Print Preview** option to view the report on-screen before printing it to paper. Unneeded columns and other individual report elements can be removed or modified to improve the appearance and usefulness of the report.

More Information:

Find step-by-step instructions for creating and customizing reports in the **Reporting Results** chapter.

Quick Export



Click the **Quick Export** button to instantly create a copy of the current screen in Excel®.

Where it works:

Wherever you see the **Quick Export** button, you can export the view. Almost every Data View has a Quick Export button. For example, the Matrix, Statistics, Cutoff and Validation tabs offer it and the Graph tab has two, one for the curve and another for the Curve Results Table.



Adjust the **Quick Export Settings** to select the target spreadsheet for the Quick Export. You can add items to the bottom of an existing Excel worksheet, for example. Select **Protocol > Protocol Options > Quick Export Settings**.

If you don't have MS Excel:

Use one of these options (described in the **Exporting Results** chapter) to select content and export it for use in another software application:

- **File Export Builder:** to export selected data to a text file
- **Right-Click Menu Options - Copy to Clipboard and Save As:** to copy or save the current selection for use in another software application

Reader System Test

System > Diagnostics > Run System Test

❖ The System Test for the **Clarity™ Microplate Luminometer** must be performed using the Clarity PC software. Refer to the Clarity Operator's Manual for instructions.

Run the Test

Most BioTek readers perform a self-test every time they're turned on, but when you want to view and/or print the results of a system (aka optics) test:

1. Select **System > Diagnostics > Run System Test**
2. When there is more than one reader attached to the PC, select the desired reader and click **OK**
3. When the test is completed:
 1. Fill in the text fields, **User, Company, Comments**, to be included in the report of the test results. Then, click **OK**.
 2. **Print** the report to retain a hard copy for your records
 3. **Save As** to convert the results to a text file. This is especially useful when troubleshooting a reader. You can email the text file to BioTek TAC.

Test History

Gen5 keeps the results of System Tests when they are performed using the menu controls. To review or print them, select **System > Diagnostics > Test History...**

Setting up an Experiment

File > New Experiment

About Experiments

In Gen5, all plates are processed in an Experiment, which is based on a protocol. The Experiment holds all the information: the **Protocol** as it was executed, the plate layout, the raw data, and the transformed data and calculation results from Data Reductions. An experiment is stored in a file with an **.xpt** extension.

How to:

1. Click the  button or select **File > New Experiment**
This opens the **Protocol** selection dialog with the most recently opened/modified protocol selected
2. Select a protocol:
 - You can select an existing one: double-click the desired protocol, or
 - Select **Default Protocol**
3. Review (and modify as needed) the elements of the selected protocol, and when you're ready click the **Read** button 
4. Select **File > Save** or click  and give the experiment file a unique name.

In an experiment, you can:

-  **Add Plates:** to process additional plates using the same protocol
- **Delete** and **Renumber Plates:** When multiple plates have been added to an experiment, highlight a plate in the menu tree, right click and select Delete. After removing a plate, right click and select Renumber All, if needed
-  **Read multiple plates:** when multiple plates have been defined in an experiment, highlight one and hold the **Ctrl** key to select others, then **right click** for options: **Read** or **Print**
-  Use the **Quick Export** feature to instantly export the current view to Excel®

 You can make changes to the Protocol when running it in an Experiment. Select **File > Save Protocol As** to save the changes for a future experiment. Otherwise, the Experiment's protocol and the original Protocol will be different.

Read a Plate



Highlight the plate in the menu tree and click the **Read** button on the toolbar or right-click and select **Read** to read the plate.

Prerequisites and other issues:

- Minimally, you must define some reading directions, like wavelength. In Gen5 this is a [Quick Read](#)
- If the reading is part of an experiment or assay that you'll perform numerous times, [create a new protocol](#)
- Click the **Read** button when an **experiment is stopped** and you want to begin again:
 - you can **Resume** a reading procedure: continue from the stopping point, if a Stop/Resume step has been defined
 - or **Re-Read** the plate, starting from scratch, i.e. overwriting any previously obtained measurements
- **Define the Runtime Prompts:** You can customize the "prompts" or text fields users see when they read a plate

Immediately before a read:

- **Runtime Prompts:** Fill in the fields defined in the Protocol as Runtime Prompts
- **Set Temperature:** Gen5 displays a warning message telling users to wait until the defined temperature is reached before proceeding with the read
- **Load Plate:** Gen5 displays the current reading-chamber temperature (if the reader has an incubator). If the temperature is too high, you can **Cancel** the read to wait until it cools down

Acquiring Data

There are three ways for Gen5™ to acquire the data used in calculations and analysis:

- Reading a plate
- Manual entry
- Import a text file

In Gen5 select **Help>Help Topics** to learn about the latter two options.

Chapter 6

Designing a Protocol

This chapter covers the steps required to create a protocol. It also provides instructions for using the Default Protocol.

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Design a Protocol

The menu tree provides a visual clue to the steps involved in creating most protocols (**File>New Protocol...** opens only the Protocol section of the menu tree):

1. [Define the Procedure \(or Reading Parameters\)](#)
2. [Define the Plate Layout](#) (for all except **Gen5 Reader Control** software)
3. [Define the Data Reduction Requirements](#) (for all except **Gen5 Reader Control**)
4. Define the **Runtime Prompts** to collect user input at runtime (plate reading)
5. [Define the Reporting Requirements](#)
6. Save the Protocol

❖ **Important:** follow this sequence of tasks, when developing a protocol to take advantage of Gen5's automatically created data reduction events. For example, when you add Blanks to the Plate Layout, Gen5 automatically creates a Blank-Subtraction data set.

You can find specific, step-by-step instructions for numerous types of protocols in Gen5's Help system.

Protocols are run or executed within an Experiment. Learn more about the differences between Experiments and Protocols in the [Essential Concepts](#) chapter

❖ Alternatively, you may want to begin with one of [Gen5's Sample Protocols](#) described in Chapter 4. They can give you a head start.

Defining the Reading Procedure

Protocol > Procedure

Set up the **Procedure** to control the reader: define the  reading parameters and related activities of the **Protocol/Experiment**.

- ❖ **Grayed out?** Once a reading has been done in an experiment, the Procedure cannot be changed for the current experiment. If this isn't the case, your System Administrator may have restricted your ability to modify the protocol elements.
- ❖ **Grayed out buttons** mean the action cannot be performed by the current reader or because previously defined steps, e.g. kinetic loop, limit the function.

How to:

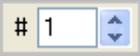
1. Click a button to add that step to the procedure. Most buttons open a screen for defining the parameters of that step, e.g. Read lets you define wavelengths, etc. When defining a kinetic or synchronized well/plate mode analysis, add the **Kinetic** or **Synchronized Mode** steps **first**. Kinetic and Synchronized Mode steps form a loop or block. Put the Read and other valid steps to be performed inside the loop, between the Start and End. Monitor Well is similar, first add the **Monitor Well** step and then, add a Read step inside the monitor-well loop.
2. Define the details of the step and click **OK**
3. Click **Validate** to check the selection and sequence of the steps
4. For all Procedures define the **Plate Type**

- ❖ More details and a Validation Checklist are provided in the **Defining a Procedure** chapter beginning on page 117.

Defining the Plate Layout

Protocol > Plate Layout

It's easy to define the plate layout with Gen5's tools for identifying samples, standards, controls and blanks. Follow these steps:

1.  In the **Well Settings** box in the top-left corner, select the **Type** of specimen
2.  Customize the **ID** or Well Identifiers, if necessary, by clicking the 3-dot button.
3.  Define the **Concentration** or **Dilution**, if applicable, by clicking the 3-dot button.
4. Assign the well IDs to their corresponding locations in the plate grid by clicking in the wells in the matrix.
 -  When you select a corresponding starting # the ID changes accordingly for assignment to the plate.
 - Use the **Auto Select** and **Replicates** options to speed up your work: set the options and click and drag to fill multiple wells at once. Click a column or row header to fill it.

- ❖ The type of plate, e.g., 96-well, is defined in the [Procedure](#) and displayed in a representative matrix or grid format in the Layout and Transformation screens.
- ❖ More details are provided in the **Plate Preparation** chapter beginning on page 211, and Gen5's Help offers an instructive animated demo of the process: Select **Help > Tutorials**.

Setting up Data Reduction

Protocol > Data Reduction

There are several options available for interpreting the results of your experiment. Gen5™ automatically creates the most commonly applied data reduction steps (based on previously-defined Protocol parameters). You can design your own or modify the calculations.

- ❖ Find more details and the **Top 6 Things You Should Know about Data Reduction** in the chapter beginning on page 235.

Data Reduction Options

Find details about each option in the Data Reduction Options chapter.

-  **Define a Transformation**
-  **Define a Curve Analysis**
-  **Define a Well Analysis**
-  **Define Cutoffs**
-  **Define Validation criteria**
-  **Fluorescence Polarization**

- ❖  **Gen5** shows an invalid data reduction step by blocking out its icon. Changing the **Procedure**, e.g. reading parameters or sequence of events, renaming a Read step or data set, or making other **Protocol** changes can invalidate a data reduction step. Generally, it is easiest to delete the invalid step and recreate it, selecting valid options.

Customizing Data Views, Reports, and Exports

Gen5™ provides several tools for changing the appearance of views and reports. All selections and customizations to views and to report and export elements can be saved with the protocol, (**File>Save Protocol As**) so they are retained for all future experiments based on that protocol.

Check out the tips and rules for customizing the views and output format of data elements:

Best Practice:

- **A good habit** to develop when setting up a Protocol is customizing the content and format of the **Data Views**: the on-screen presentation of data. The settings defined in Data Views become available for selection in the Report Builder and Export definition screens, so it is most efficient to **begin with Data Views** (after defining the Procedures, Plate Layout, and Data Reduction details)
- **Important:** Attributes applied to data items and field groups in the Data Views dialog take effect going forward. They do not replace, update, or overwrite an item that has previously been assigned to a Report or Export output. You must refresh the Report/Export contents after making any changes to a data element, to capture them in the report. *Any previously saved Experiment will not reflect the content or formatting changes.*
- **Gen5 limits the ability to customize system-provided views**, but creating new ones offers enormous flexibility. For example, if you do not use Sample IDs, you'll always have an empty Name column in the system-provided Statistics tables. But, you can **create your own view** that excludes the Name/Sample ID data point.
- **You can choose** the way Gen5 formats data/text that is too long to fit completely in a field or column by **Changing the Font** settings for a data elements.
- Review other related rules: **Customizing Reports**
- **Field Groups** (for use in reports) can only be created in the Data Views dialog, but they can be modified in the Report Builder and Export dialogs

Reporting Results

Gen5 offers several tools for reporting results from experiments.

You can use:

- Gen5's full-featured report engine: begin with the [Report Builder](#) described in the **Reporting Results** chapter beginning on page 323.
- or [Export](#) the results and use Excel® or another software application to generate a report; its described in the **Exporting Results** chapter.

Using the Default Protocol

Use the default protocol to work more efficiently in Gen5.

About the Default Protocol

System > Preferences > Default Protocol...

Gen5 provides a mirror image of the Protocol menu tree to create a **Default Protocol**, a template, that helps you save time when creating new Protocols. If some protocol elements, like Plate Layout, remain the same from experiment to experiment in your lab, you can make them default settings that are applied to all new protocols. Any aspect of the **Default Protocol** can be overridden in a newly created protocol or experiment using the regular menu options.

If it hasn't already become obvious, you'll quickly learn that defining the [Procedure](#) or read parameters is a prerequisite to defining Data Reduction and selecting Report and Export content. While this fact generally limits the role of the Default Protocol, it can still be used to define numerous settings that you're likely to apply to all experiments.

The Default Protocol is stored in [Gen5's database: SharedDB](#). When this is stored on a network drive that all your Gen5 users can access, they can also share the Default Protocol.

More on the Default Protocol Settings

Unless the reading parameters, like wavelengths/filter sets, remain the same from experiment to experiment in your lab, it is best to **NOT** define a Procedure and Data Reduction steps for the Default Protocol. Users can alter the Procedure when they define a new protocol, but this action will invalidate the Data Reduction steps, which may go unobserved by the user.

Customize Well IDs

A major advantage of the Default Protocol is to customize the Well IDs (see next page) used in the Plate Layout. You may also be able to define the concentration values and location of Standards, and Blanks on the plate. Similarly, for Sample Identification fields. The customized IDs become available for selection in new protocols. Find instructions for defining customized Well IDs and Sample Identification fields in the Plate Preparation chapter beginning on page 211.

Common Default Settings

You may want to define **Runtime Prompts**, **Report Headers** and **Footers**, and **Export Options** like file storage locations, which are commonly static elements in an organization's protocol design.

❖ **Important:** Defining the default protocol settings takes effect going forward, i.e. they are only applied to newly created protocols and have no effect on existing protocols or experiments.

Setting up the Default Protocol

System > Preferences > Default Protocol...

Use these controls to define the default settings **for newly created protocols**.

How To

Define the Default Protocol as you would a standard protocol, keeping in mind that your selections will be applied to all newly created protocols.

 As with standard protocols, you cannot define Data Reduction steps until the reading Procedure has been defined. Conversely, altering the Procedure can invalidate any previously defined Data Reduction steps.

Reset

Use the **Reset** button to clear the Default Protocol settings, erasing any selections or customizations. Reset returns the Default Protocol to its out-of-the-box definition.

Customizing IDs in the Default Protocol

Gen5 ships with certain abbreviations for the various well types, but they may not match your organization's naming convention. Customizing the Well IDs in the Default Protocol's Plate Layout makes them available for all newly created protocols. This can be a real timesaver for your users.

Common Changes

Well Type	Default ID	New Default ID
Sample	SPL	SMP
Assay Control	CTL1	PC (Positive Control)
	CTL2	NC (Negative Control)
	CTL3	HPC (High Pos)

Chapter 7

Defining the Procedure

This chapter provides instructions for setting up Gen5's Procedure, the reading parameters.

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Defining the Reading Parameters

Protocol > Procedure

Set up the **Procedure** to control the reader: define the  reading parameters and related activities of the **Protocol/Experiment**.

- ❖ **Grayed out?** Once a reading has been done in an experiment, the Procedure cannot be changed for the current experiment. If this isn't the case, your System Administrator may have restricted your ability to modify the protocol elements.
- ❖ **Grayed out buttons** mean the action cannot be performed by the current reader or because previously defined steps, e.g. kinetic loop, limit the function.

How to:

1. Use the drop-down list to define the **Plate Type**
2. Click a button to add that step to the procedure. Most buttons open a screen for defining the parameters of that step, e.g. Read lets you define wavelengths, etc. When defining a kinetic or synchronized well/plate mode analysis, add the **Kinetic** or **Synchronized Mode** steps **first**. Kinetic and Synchronized Mode steps form a loop or block. Put the Read and other valid steps to be performed inside the loop, between the Start and End. Monitor Well is similar, first add the **Monitor Well step** and then, add a Read step inside the monitor-well loop.
3. Define the details of the step and click **OK**
4. Click **Validate** to check the selection and sequence of the steps

- ❖ Gen5 validates the Procedure when you save and close the Procedure.

Features:

- You can **Drag and Drop** steps in the Procedure to change their sequence order
- Highlight a step in the Procedure, and then click an action button to add a step before it
- Double click a step to open it for editing
- Select a step in the sequence and right click for additional options
- Click **Validate** at any time to verify the reader's ability to perform the current sequence of steps
- Highlight a step and press Delete to remove it from the procedure

- ❖ Drag and Drop is limited in Synchronized Modes, for example, you cannot drag and drop a step into or out of a Well Mode block

Review the [Validation Checklist](#) on page 121

Learn about: Synchronized Modes (Synergy 2, Synergy HT and FLx800 with Injectors) in the **Fluorescence and Luminescence** chapter.

About Gen5's StepWise Procedure

Gen5™ offers lots of flexibility in defining a **Procedure**: the read steps and related activities, like incubation, shaking (or mixing), dispensing reagent, and so on. Each activity or requirement is defined chronologically or **StepWise**.

The StepWise Procedure can be simple, performing only one reading at one wavelength. It can also be complex, a series of events that includes multiple readings, incubation, shaking, and ejecting plates between reads to add reagent. The sequence of steps in the **StepWise Procedure** workspace defines the order of events performed by the reader.

The current reader determines the availability of options:

- Readers are set up under **System>Reader Configuration**.
- **Grayed-out buttons?** One of three reasons:
 - The reader cannot perform the function; possibly due to previously-selected events like Kinetic
 - Your System Administrator has restricted your rights to modify the Procedure
 - A plate has already been read in this experiment
- When more than one reader is connected to the PC, Gen5 opens the **Instrument Selection** dialog to let you select the desired reader before offering the Procedures dialog.

Depending on your reader, the possible combination of steps in a **Procedure** is numerous. For example, you can:

- **Set the Temperature** followed by a **Delay** to reach the correct setting, before adding a multiple-wavelength **Read** step
- Give the plates a long time to react in an experiment by adding a **Stop/Resume** step between readings. This frees up the reader for use in other experiments during the down time
- Perform multiple **Read** steps, each interspersed with a **Plate In/Out** to add reagent, followed by a **Shake** step to mix the contents before performing the next read
- Define a kinetic analysis: Click **Kinetic**, followed by a **Read** step. Gen5 adds an **End Kinetic** event to close the kinetic loop
- Apply different read methods within a Procedure, performing a **Scanning** step, followed by a multi-read **Endpoint** step, and finish with a kinetic loop

- Define one **Read** step for **half the plate**, and another Read step (with different parameters) for the other half of the plate
- Perform a multi-mode or **multi-detection** experiment by defining **Absorbance** and **Fluorescence Read** steps in the same Procedure
- and so on ...

 Along with the reader's capabilities, the sequence of a step in the **Procedure**, especially the steps immediately before and after it, determines its validity. Click **Validate** to test the sequence of steps.

Validate the Procedure

About Procedure Validation

Gen5™ supports your effort to design a protocol by **validating** the **Procedure** based on the capabilities of the current microplate reader and the sequence of steps to be performed. Validation is also helpful at runtime, when a protocol may have been designed without a specific reader attached to Gen5, and the actual reader's capabilities are more limited than expected by the protocol. Gen5's validation feature will display an error message alerting you of the need to fix the Procedure.



Click the **Validate** button in the **Procedure** dialog to validate the reading events. Certain activities in the Procedure sequence, like Shake, cannot be standalone events, but must be related to a read or other activity. Here are the rules:

Device Step	Valid Combination/Limitations
Read	May be a standalone event. At least one read step is required.
Set Temperature	May be a standalone event Cannot be inside a Kinetic loop Cannot be in a synchronous block
Shake These restrictions apply to all readers except for the Synergy 2 , which supports a shake step anywhere in the Procedure.	Shake » Read Shake » Start Kinetic » Read » End Kinetic Start Kinetic » Shake » Read » End Kinetic Shake cannot : <ul style="list-style-type: none"> • precede a Spectral or Area Scan • be included in a Well Mode block • be the first step in a multi-detection kinetic loop if the first read is Luminescence
Dispense	May be a standalone event
Delay	May be a standalone event Cannot be in a kinetic loop Cannot be between Shake and Read
Kinetic	Requires at least one read step Start Kinetic » Read » End Kinetic Shake » Start Kinetic » Read » End Kinetic Start Kinetic » Shake » Read » End Kinetic

Device Step	Valid Combination/Limitations
	Cannot include Scanning and Spectral reads Only one read step allowed in Synchronized Well Mode Only one read and one shake step allowed in Synchronized Plate Mode
Monitor Well	Monitor Well » Read » End Monitoring Monitor Well » Shake » Read » End Monitoring Shake » Monitor Well » Read » End Monitoring
Plate In/Out	May be a standalone event Cannot be inside a Kinetic loop Cannot be in a synchronous block
Stop/Resume	May be a standalone event Cannot be inside a Kinetic loop Cannot be in a synchronous block Cannot be the final event in a sequence
Well Mode	Requires at least one read step Synergy HT: can have up to 9 steps (excluding Delay) Synergy 2: can have up to 20 steps (excluding Delay) FLx800: can have up to 3 steps (excluding Delay) Kinetic loop is limited to one read, except Synergy 2 allows a Shake step Multi-Detection kinetic loop is not allowed
Plate Mode	Requires at least one read step Synergy HT: can have up to 9 steps (excluding Delay) Synergy 2: can have up to 20 steps (excluding Delay) FLx800: can have up to 3 steps (excluding Delay) Kinetic loop is limited to one read and one shake Multi-Detection kinetic loop is not allowed

Read Step

Protocol > Procedure > Read

Define the reading parameters based on the capability of the current reader:

1. (Optional) Enter a **Step Label** or unique name for this step. Data sets based on the reading results will use the label in online views, reports, and export files.
2. Keep the **Full Plate** or set a portion of the plate to process. (The Plate Type is set for all steps in the Procedure.)
3. Select the **Detection Method**. Options are controlled by the current reader.
4. Select the **Read Type**. Options are controlled by the current reader and the detection method selected above.
5. Select the **Read Speed** from the list offered for the current reader
6. Set the **Wavelengths** or **Filter Sets**:
 - 1 Use the numbered buttons to set the number of wavelengths/filter sets to obtain measurements with. Kinetic, Spectrum, Area and Linear Scans limit this option.
 - 2 Click the down arrow or type in the text field to set the wavelengths
7. If applicable, define:
 - Pathlength Correction
 - Optics Position
 - Sensitivity and Filter Set Options
 - Top Probe Vertical Offset

Read Types

Depending on the reader, detection method, Gen5 product level, and the type of analysis you're conducting, one of several read types can be selected:

- **Endpoint**
 The most commonly used Read Type, Endpoint, performs one read in the center of the well for each wavelength. It is the only read type that supports Pathlength Correction.

 Check your assay kit instructions to determine if this type of reading is required. Endpoint reads are generally conducted after a **Stopping Solution** is applied to the samples or when the effects of the chemistry occur at an expected time point.

- **Area Scan**

When performing an **Area Scan**, the reader takes multiple measurements down and across each well, in a “matrix” format. This method is more effective for cellular assays than reading once in the center of the well.

- Learn more in the **Scanning Analysis Options and Features** chapter

Readers that support Area Scanning include the ELx800, μ Quant, FLx800, SynergyHT, and Synergy 2.

❖ **Note:** the Synergy 2's probe size limits its ability to perform Fluorescence area scan in plates with a small well diameter. Generally, this means you must use a plate with fewer than 96 wells.

Read Matrix Size represents the number of measurements taken across and down each well. If, for example, the Read Matrix Size is 5 x 5 a total of 25 measurements are taken. The potential Read Matrix Size is a function of the well size of the current plate.

- **Linear Scan**

When performing a **Linear Scan**, the reader takes multiple measurements in a **line** across the center of each well. Linear scanning allows you to observe a pattern that may be present in the well bottom, such as an agglutination pattern.

- Learn more in the **Scanning Analysis Options and Features** chapter

Readers that support linear scanning include the ELx808 and all PowerWave models. Note for PowerWave X Select: Linear scanning is supported for the 96-well plate type only.

Horizontal Reading Points setting represents the total number of points to be read across the center of each well. Valid entries are odd integers from 1 to 39.

❖ **Note:** If the Scanning options are inaccessible, well scanning cannot be performed with the currently defined plate type. This may be due to a hardware limitation or an unacceptable combination of optic probe size and well diameter.

- **Spectrum**

During a **Spectrum Read**, multiple readings are taken across a wavelength range. The objective is to plot a graph with absorbance versus wavelength.

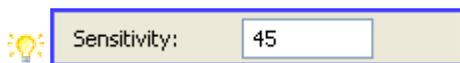
- Learn more in the **Scanning Analysis Options and Features** chapter
- The **Stop** wavelength must be greater than or equal to the **Start** wavelength + the **Step**

Readers that support spectrum reads are μ Quant, and all models of the PowerWave, Synergy HT and Synergy 2.

Read Step for Fluorescence for FLx800 and Synergy HT

❖ Synergy 2 users find instructions on page 127

Protocol > Procedure > Read



When defining reading parameters for Fluorescence analysis, setting the **PMT Sensitivity** (for the **Filter Sets**) is important for obtaining useful measurements. The valid range is 25 to 255, but too low a setting, like 25, can result in insufficient readings, and too high a setting, >120, can damage the PMT. BioTek recommends a setting between **40 -120** for Fluorescence assays and between 150 - 255 for Time Resolved Fluorescence.

1. (Optional) Enter a **Step Label** or unique name for this step. Data sets based on the reading results will use the label in online views, reports, and export files.
2. Keep the **Full Plate** or set a portion of the plate to process. (The Plate Type is set for all steps in the Procedure.)
3.  Click the down arrow at **Detection Method** to select **Fluorescence**
4. Select **Time Resolved** to perform this type of fluorescence analysis (learn more in the **Florescence and Luminescence** chapter)
5. In Synchronized mode, you can select **Close Light Shutter** to turn off the light between reads. Optionally, to protect the fluorescent nature of your samples, use this feature to block the light between measurements to prevent photo-bleaching effects. Gen5 blocks the light with a Plug in the filter wheel.

Important:
A plug or blocking filter in the excitation filter wheel must be adjacent to the filter used in the reading. Two plugs must be placed next to each other (which ensures they are adjacent to the two filters used) in a dual-filter-set read step. Define the Reader Settings
5. For the **Read Type** select:
 - [Endpoint](#)
The most common read type, Endpoint, performs one reading per well for each filter set defined.
 - [Area Scan](#) (not available in Synchronized mode)
When performing an **Area Scan**, the reader takes multiple measurements down and across each well, in a “matrix” format. This method is more effective for cellular assays than reading once in the center of the well.
6. Set the **Filter Sets**:
 - 1  Use the numbered buttons to set the number of wavelengths.

- 2 Click the down arrow to select the filter
- 3 If applicable, define:
 - **Optics Position**
 - **Sensitivity** or click **Options** to let Gen5 determine the optimal setting
 - **Filter Switching**: reading each well with both filters before moving to the next well, is offered when only two filters are selected.

❖ In **Synchronized mode**, the read step settings for the first read in a Plate or Well mode block are applied to any subsequent read steps in the block.

Learn more in the **Florescence and Luminescence** chapter

Read Step: Synergy 2 Fluorescence

Protocol > Procedure > Read



Sensitivity:

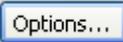
When defining reading parameters for Fluorescence analysis, setting the PMT **Sensitivity** (for the **Filter Sets**) is important for obtaining useful measurements. The valid range is 25 to 255, but too low a setting, like 25, can result in insufficient readings, and too high a setting, >120, can damage the PMT. BioTek recommends a setting between **35 -120** for Fluorescence assays and between 100 - 160 for Time Resolved Fluorescence (TRF).

1. (Optional) Enter a **Step Label** or unique name for this step. Data sets based on the reading results will use the label in online views, reports, and export files.
2. Keep the **Full Plate** or set a portion of the plate to process. (The Plate Type is set for all steps in the Procedure.)
3.  Click the down arrow at **Detection Method** to select **Fluorescence**
4. Optionally, select **Time Resolved** or **Polarization** to perform this type of fluorescence analysis. Your choice enables or disables related options as appropriate for the process.
5. **Read Speed:** Use the drop-down list to make a selection and/or click the 3-dot button to change the default settings for **Measurement Options**
6. For the **Read Type** select:
 - **Endpoint:** The most common read type, Endpoint, performs one reading per well for each filter set defined.
 - **Area Scan:** (not available in Kinetic or Synchronized mode): When performing an **Area Scan**, the reader takes multiple measurements down and across each well, in a “matrix” format. This method is more effective for cellular assays than reading once in the center of the well. Also, The Synergy 2's probe size limits its ability to perform Fluorescence area scan in plates with a small well diameter. Generally, this means you must use a plate with fewer than 96 wells.
7. **Light Source:** except for TRF, you can select the lamp to use for this read step: Xenon Flash (Xe) or Tungsten (Tg)

Using the Xenon Flash (Xe)	
Advantages	Disadvantages
Enables Sweep mode as a Read Speed	Prohibits use of the Extended Range
Very high energy, slightly more sensitive than Tg bulb	It is expensive compared to the Tg

High light output below 300 nm (UV Fluorescence)	Noise
Performs direct protein and amino acid quantification assays	

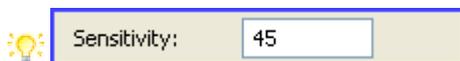
Using the Tungsten Lamp (Tg)	
Advantages	Disadvantages
Inexpensive, with high sensitivity for Fluorescence Intensity (FI) and Fluorescence Polarization (FP)	Sweep read speed is prohibited
Enables Extended Dynamic Range	Cannot perform TRF
Strong and stable light output in visible range	Slightly less sensitivity than Xe Flash
	No light output below 300 nm

8. In Synchronized (non-kinetic) mode, you can select **Close Light Shutter** to turn off the light between reads. To protect the fluorescent nature of your samples, use this feature to block the light between measurements to prevent photobleaching effects. Gen5 blocks the light with a Plug in the filter wheel.
9. Set the **Filter Sets**:
10.  Use the numbered buttons to set the number of wavelengths
11. Click the down arrow to select the filter
12. If applicable, define:
 - Optics Position: for top reading select the mirror
 - **Sensitivity** or click  to let Gen5 determine the optimal setting
 - **Filter Switching**: reading each well with both filters before moving to the next well, is offered when only two filters are selected.
 - Top Probe Vertical Offset

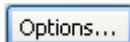
- ❖ In Synchronized mode, the read step settings for the first read in a Plate or Well mode block are applied to any subsequent read steps in the block.
- ❖ The Filters offered for selection are defined by the Filter Wheel Library or Reader Configuration

Read Step for Luminescence

Protocol > Procedure > Read



When defining reading parameters for Luminescence analysis, setting the **PMT Sensitivity** is important for obtaining useful measurements. The valid range is 25 to 255, but BioTek recommends a setting between **100 - 160** for Luminescence assays.

1. (Optional) Enter a **Step Label** or unique name for this step. Data sets based on the reading results will use the label in online views, reports, and export files.
2. Keep the **Full Plate** or set a portion of the plate to process. (The Plate Type is set for all steps in the Procedure.)
3.  Click the down arrow at **Detection Method** to select **Luminescence**
The **Read Type** must be set to Endpoint
4. Enter the **Integration Time**: to set the read duration for each well in seconds or milliseconds. Click in the field and enter the Sec.Msec or use the spin buttons to set the duration.
Valid values:
 - Synergy HT: 0.1 - 19.9 seconds;
 - Synergy 2: 0.1 - 99.9 seconds, in 20 ms intervals
 - FLx800: 0.1 - 6.0 seconds
5. **Synergy 2**: Click the 3-dot button to change the default settings for **Measurement Options**
6. Set the **Filter Sets**:
 - 1  Use the numbered buttons to set the number of wavelengths.
 - 2 Click the down arrow to select the filter or **Hole** (to not filter the light)
 - 3 If applicable, define: (Learn more in the **Florescence and Luminescence** chapter)
 - **Optics Position**
 - **Sensitivity** or click  to let Gen5 determine the optimal setting

❖ **In Synchronized Mode**, the read step settings for the first read in a Plate or Well mode block are applied to any subsequent read steps in the block.

Read Step for Spectrum Analysis

Protocol > Procedure > Read

Define the reading parameters based on the capability of the current reader:

1. (Optional) Enter a **Step Label** or unique name for this step.
2. Keep the **Full Plate** or set a portion of the plate to process.
3. Set the **Detection Method** to **Absorbance**
4.  Click the down arrow to set the **Read Type** to **Spectrum**. Options are controlled by the current reader — if Spectrum is not available, the current reader does not have this capability.
5. Set the range of **Wavelengths**:
Acceptable Values:
 - The acceptable range for the **Start wavelength** is from the lowest wavelength the reader supports to one less than the Stop wavelength selected.
 - The acceptable range for the **Stop wavelength** is any wavelength greater than the Start wavelength to the highest wavelength allowed by the reader.
 - The acceptable range for the **Step** value is any number equal to or less than the difference between the Start and Stop values.
 - The only **read speeds** available for Spectrum reads are Normal and Sweep.
6. Enter the **Start** and **Stop** wavelengths to define the spectral range
7. Enter the number of **Steps** to define the number of measurements to take
8. Set:
 - **Read Speed** from the list offered for the current reader
 - **Calibrate Before Read**: When selected, the reader will always perform calibration at the wavelengths specified in the protocol, just prior to plate reading. If Calibrate is not selected, the reader will calibrate at only those wavelengths specified in the protocol that have not yet been calibrated since the reader was turned on.

Procedure Steps: Reading-Related Activities

Protocol > Procedure

The following features may or may not be available for creating a protocol depending on the attached reader, and your level of software.

- **Set Temperature** (Incubation): page 131
- **Shake** the plate: page 132
- **Dispensing Reagent**: page 131
- **Kinetic Analysis**: page 135
- **Delay Step**: page 136
- **Monitor Wells**: page 137
- **Plate In/Out**: page 138
- **Stop/Resume** the experiment: page 139
- **Synchronized Modes**: refer to the **Fluorescence and Luminescence** chapter



Set Temperature

Use these controls to set the desired temperature for the reading chamber or incubator. Most BioTek readers allow a temperature range of 20-50°C. Add a **Delay** step to the Procedure to incubate the plate. **Learn more below.**

1. Enter the desired temperature in the **Temperature** field.
2. Select (or de-select) the option to **Preheat before continuing with next step** to wait for the temperature to reach the set point before proceeding with the next step in the **Procedure**. Selecting this option activates the Pre-Heating function available from the Control Panel: System>Control Panel.

- ❖ **Stop/Resume**: Gen5 considers a Stop/Resume step the end point of the series of steps that precede it. It frees up the reader for running other experiments. If you want the steps following a Stop/Resume to be performed at a certain temperature you must add a Set Temperature step after the Stop/Resume step.
- ❖ You **cannot** put a Temperature Step inside a kinetic loop
- ❖ When the experiment is started (the Read button is pressed) before the reader has reached the defined temperature, Gen5 offers the option to override the Set Temperature step.

 **Reporting the temperature:** you can include the temperature of a read step in a report or export file. Add the **Fields** to your report: from the **Plate Information** category, select the desired Temperature field.

How to incubate the plate:

The Set Temperature step in Gen5's StepWise Procedure does not by itself incubate the plate. It heats up or cools down the reader to the defined setting. To use your reader to incubate the plate define the Procedure this way:

1. **Set Temperature** with preheating
2. **Plate Out/In** step, enter **Incubate Plate** in the Comments field
3. **Delay** for the incubation duration
4. **Read**

Shake the Plate

Use these controls to set the **Intensity** and **Duration** of a Shake step to mix the plate contents:

- **Intensity** - use the drop-down list to select a level. The reader's operator's manual may define the specifications of each level.
- **Duration** - enter a time period (minutes:seconds) to shake the plate. The potential range is 1 second to 60 minutes.
- **Continuous Shake** - within a kinetic loop, if the reader is capable of performing it, you can select this option to shake the plate whenever it is not being read during the kinetic time interval

As part of a **Kinetic Analysis**:

- To shake the plate only before the **first** reading in a kinetic analysis define the sequence of activities: Shake » Start Kinetic » Read » End Kinetic
- To shake the plate before **every** reading in a kinetic loop define the sequence of activities: Start Kinetic » Shake » Read » End Kinetic
- To continually shake the plate whenever it is not being read, select **Continuous Shake** instead of a time interval

❖ In multi-mode kinetic analysis a Shake step cannot precede the kinetic loop, but can be the first step in the loop: Start Kinetic » Shake » Read » Read » End Kinetic



Dispensing

Reader models, Synergy™ and FLx800™ with injection capability, can dispense reagent to wells in accordance with several user-defined settings.

The Dispense dialog fields are described here and information to help you choose the optimal Dispense Rate begins on page 134.

Dispenser

Select the number of the Dispenser to be used for this step. The numbers correspond to the numbers on the dispense module.

Partial or Full Plate?

The button to define a full or partial plate is dynamic, it changes from **Full Plate** to a plate-map description of the area to be dispensed to/read, e.g. **A1..H5**. Click the button to define the specific wells for dispensing, and if this step is in a loop, for reading in the subsequent Read Step(s).

❖ **In Synchronized Mode** the first step in a block controls the full or partial plate for the entire block. For example, if the steps in a Well Mode block are Dispense>Delay>Read, when the Dispense step is defined to dispense to the first two columns of the plate, the Read step is also limited to the first two columns. Likewise, if the steps in a Plate Mode block are Read>Dispense>Read, and the first Read step is a partial plate read E5...H12, the Dispense and Read steps to follow will automatically be set to the same portion of the plate

Tip Prime

Priming: select an option:

- **none:** Do not prime the tip
- **before the dispense step:** Prime the tip with fluid before injection of fluid to the plate
- **once before the Well/Plate Mode block:** In Synchronized mode, you can prime the tip one-time-only before the process begins
- **before this dispense step:** In Synchronized Plate Mode, you can prime the tip before each dispense step in the block
- [More About Tip Priming:](#) Priming is performed in a small, removable priming trough located in the rear of the carrier. The purpose of tip priming is to compensate for any fluid loss at the dispense tip due to evaporation since the last dispense. Each trough holds up to 1500 µl of liquid and must be periodically emptied and cleaned. Gen5 warns you to empty the trough before the first Dispense event and keeps track of the amount of fluid primed into the trough during the Procedure. Selecting **Yes** at one of Gen5's messages to empty the trough resets the trough volume record to zero.

- ❖ Do not perform Tip Priming when using tall plates. Generally, plates with fewer than 96 wells are too tall for error-free tip priming, and it is rarely required for these larger-volume plates.

Volume: enter the amount of fluid in microliters. The valid range is 5 to 20.

- ❖ **Important:** For optimal dispense accuracy and precision when dispensing volumes less than or equal to 20 μl /well, BioTek recommends priming the tip: use a tip prime volume equal to the dispense volume. For dispense volumes greater than 20 μl /well, we recommend a tip prime volume of 20 μl .

Dispense

Volume: Enter the dispense volume in microliters. The valid range is 5 to 1000

- ▼ **Rate:** Select the dispense rate (microliters per second) based on the volume.

 **Keep the fluid path clean:** You'll get the best results from your BioTek reader with Injectors if you keep the fluid path clean. Minimally, you should **Purge** the reagent and flush the lines with DI water when the experiment is finished. Follow instructions for daily maintenance in your Operator's Manual.

- ❖ When the **Dispense** step is the last step in the Procedure, add a **Plate Out** step to eject the plate carrier when the Procedure is finished.

Dispense Rate

Gen5's options are:

Rate ($\mu\text{l}/\text{sec}$)	Volume Range (μl)
225	5-1000
250	15-1000
275	25-1000
300	30-1000

The maximum volume for a dispense operation is 1000 μl . The minimum volume depends on the dispense rate. For example, when dispensing $\leq 10\mu\text{l}$ you must use a rate of 225.

Here are some factors to consider when selecting the dispense rate or injection speed:

- Use the fastest rate for the best mixing affect in the wells
- Use the slowest rate (225) when a cell layer is involved; especially if the level of liquid in the well is low ($<100\mu\text{l}$) before injection

- Lower the rate if you notice some spills on the plate after injection. (This may be caused by a high (>200µl) or low (<50µl) pre-injection volume, or to a specific plate type, like round-bottom wells.)
- Assay kits generally provide guidelines, review the kit insert to determine its recommendation for a slow or high dispense speed
- High-viscosity fluids perform better with a slow rate

Kinetic

❖ Learn more in the **Kinetic Analysis** chapter beginning on page 177.

To set up a kinetic (or time-course) analysis, put one or more Read steps within the **Start Kinetic** and **End Kinetic** loop. The steps are conducted within the specified timelines:

Run Time: Enter the full duration of the kinetic analysis, i.e. the length of time required to perform all the steps within the kinetic loop:

- Time Format: Hours (HH): Minutes (MM): Seconds (SS) in standard mode and Minutes (MM): Seconds (SS): Milliseconds (ss) in synchronized mode.
- Maximum time period is 168 hours except in Synchronized Modes. Well Mode kinetic run time is limited to 60 minutes and the block must be completed in 2.78 hours. Plate Mode kinetic run time is limited to 24 hours and the block must be completed in 27.8 hours.

Interval: Enter the desired time interval between readings or select **Minimum Interval**. Gen5 will read the plate at every interval, e.g. every 00:10:30 = 10 minutes and 30 seconds, for the duration of the Run Time

Minimum Interval: Select this option to let Gen5 determine the fastest possible processing time. Follow the instructions in the **Kinetic Analysis** chapter.

Reads: Gen5 calculates the number of reads from your input of Run Time and Interval. The maximum number of reads depends on your reader and detection method.

❖ **Important:** Numerous factors affect the acceptable kinetic run time. To make sure your reader can process the steps in the kinetic loop within the defined timelines, click **Validate** (with the reader attached and communicating with Gen5) when all the Procedure steps have been defined. If the interval is too short for the parameters chosen, an error will be displayed.

❖ **Discontinuous Kinetic Interval:** Gen5 let's you perform a discontinuous-interval analysis that requires long periods of downtime (for rest, manipulation, or incubation) between reads. Engage this feature with the Procedure's **Advanced Options**.

❖ **Note:** The actual runtime of a kinetic loop may exceed the defined **Run Time** by +1 **Interval**. Gen5 does this to ensure that the number of **Reads** displayed are actually captured.

Delay the Procedure

Use this command to add a **Delay** event to the series of steps defined, telling the reader to halt processing for the defined duration. You cannot put a Delay step in a kinetic loop, nor can you have a Delay between Shake and Read steps.

Delay Time: Enter a time period for the duration: HH:MM:SS

 You may want to use a [Stop/Resume](#) step or [Plate In/Out](#) instead

Delay in Synchronized Mode

Use these controls to add a **Delay** event to the steps in a Synchronized Mode block, telling the reader to halt processing for the defined duration.

Delay Time: Enter a time period for the duration

- In Plate mode: HH:MM:SS (hours:minutes:seconds)
- In Well mode: Min:Sec.Msec (minutes:seconds:milliseconds)

Start Delay From: Select an option

- **Beginning of Previous Step:** to define the delay time to include the time it takes to perform the previous step, as well as the delay time
 - **End of Previous Step:** to set the delay time to begin when the previous step is finished. This is the default and most typical setting
-

 **Example:** You may want to use the Start Delay from Beginning of Previous Step option in a Dispense>Wait>Read protocol when the precise timing of every step is critical to your experiment.

 **FLx800:** In Well Mode it is necessary to include a Delay step set to "Start delay from Beginning of previous step" to precisely control the reader to process each well within the same timeline. Set the Delay Time sufficient to perform the previous step with a little padding: this is usually 0:00.10 milliseconds or more. When you click the Validate button, Gen5 communicates with the FLx800 to determine if the Procedure is doable. If not, it provides instructions for adding a Delay step or increasing the Delay Time.



Monitor Wells

Use these controls to define well-monitoring criteria that must be met before the reader begins to capture the actual measurement values used in the experiment.

About Monitoring Wells

Gen5's Monitor Wells feature can be used to detect a certain level of activity in the plate before capturing regular, saved measurements. You define measurement criteria that must be met in one or more specific wells before the complete plate read can begin. Gen5 directs the reader to continuously read the wells at the defined time interval until the criteria are met. This feature sets up a loop, like a Kinetic loop, with a start step and an end step in the Procedure. A Monitor Read step must be defined within the loop. A Shake step is also permitted either before the read or before the entire Monitor Well loop.

Example: you can specify that the average of the measurements in wells A1, A2, B1 and B2 must be greater than or equal to 0.500 OD before initiating the plate read. When the Monitor Wells step is executed, the following information will be displayed: wells being monitored, current value of the wells, the required measurement value, and the elapsed time since the last well was read. At any point the user can choose to bypass the monitoring process and continue forward with the read or cancel the operation, aborting the entire Procedure.

How To:

- Click **Monitor Well** to start the loop
 - Interval:** enter the time interval between readings for the duration of the well monitoring. Alternatively, check the **Minimum Interval** option (if you have a reader attached) to let Gen5 determine the shortest possible interval for monitoring
 - Stop Monitoring When:** tells the reader when to conclude the monitoring process. Use the drop-down list to select a conditional option and an operator, and enter a measurement value to be met:

Condition	Operator	Measurement Value
At least one well	> greater than, >= or equal to	Absorbance valid range: – 1.000 to 5.000
Average of wells	< less than, <= or equal to	Fluorescence/ Luminescence valid range: 0 – 99998
All wells		

- Define a read and shake step, if desired, for the Monitor Well loop:
 - Read:** with End Monitoring highlighted in the Procedure, click **Read** and define the **Wells to Monitor:**
Click to select the wells to monitor for the "Stop Monitoring

When" criteria. Single or multiple wells can be selected by clicking in the grid. Multiple wells must be contiguous.

- Define the reading parameters using the modified Read step dialog. .
 - Add a **Shake** step, if desired, as normal.
3. Add the regular Procedure steps to follow the **Monitor Well loop**, i.e. an endpoint or kinetic reading and any associated activities. Gen5 always requires at least one actual read step. The "Monitor Read Step" does not fill this requirement.

 **Important:** Gen5 does not save the reading results (measurements) obtained during the Monitor Well step. Only measurements collected during a regular read step are retained for analysis and data reduction.

Plate In/Out

Add a **Plate In/Out** step to a **Procedure** to pause the run and change the position of the plate carrier. This command can be used to perform a manual task, like adding reagent to the plate between reads steps, for example. You can also use this step to change the behavior of the plate carrier, for example, if you want to move the carrier back into the reader at the end of a run.

❖ Unlike the **Stop/Resume** step, this command does not completely interrupt or end the run.

Select one:

- **Move carrier out**, Display dialog, Move carrier in

Comment:

Enter text in the **Comment** field. When the plate is ejected Gen5 will display the comment.

- **Move carrier out** (no dialog)
- **Move carrier in** (no dialog)

Results:

Move carrier out, Display dialog, Move carrier in:

When the first option is selected, Gen5 displays the comment in a dialog like this when the plate carrier is moved out:

When the user clicks **OK**, the plate carrier is moved back in. The plate can be handled in the interim.



The other two options open or close the plate carrier without displaying a message.



Stop/Resume

Use this command to stop the experiment where it is inserted in the Procedure. It is typically used for long interruptions (such as incubation periods), and allows the reader to be used for other experiments. Gen5 will stop the experiment until you resume it. [See how to resume below.](#) Only then will it continue to perform the Procedure steps that follow the Stop/Resume step. You can close the experiment when it is stopped and reopen it when you're ready to resume.

- ❖ **Set Temperature:** Gen5 considers a Stop/Resume step the end point of the series of steps that precede it. If you want the steps following a Stop/Resume to be performed at a certain temperature you must add a Set Temperature step after the Stop/Resume step.

Set up:

Eject Plate Before Stop: Gen5 will open the plate carrier to eject the plate before stopping the experiment, unless you de-select this option. When this option is not selected, Gen5 lets the previous Procedure step determine the position of the plate carrier. Since most Procedure steps do eject the plate upon completion, to keep the plate inside the reader during the downtime, you may need to add a Plate In step before the Stop/Resume step.

Comment: Enter a comment to be displayed on-screen

Results:

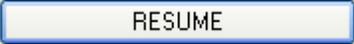
The Comment you enter, "Add reagent" in this example, is displayed in a message like this when the experiment is stopped. Users must click **OK** to acknowledge the message. The experiment is stopped, until it is resumed.



Gen5 signals the experiment has been stopped using the plate-paused icon in the menu tree.



To Resume:

1. Open the experiment, if it was closed.
2.  Click the **Read** button when you're ready to resume the experiment. The standard read screen changes, offering **Resume** instead of Read.
3.  Click the **Resume** button to continue with the Procedure. Note: If **Re-Read** is selected, Gen5 restarts the Procedure from its beginning (first step), reading the plate again and discarding the initial data.

Chapter 8

Fluorescence and Luminescence

This chapter provides useful information for conducting Fluorescence and Luminescence analysis, including Time-Resolved Fluorescence. For Synergy HT and Synergy 2 readers, there is also a section on Multi-Mode assays. You'll find details about how to set the PMT Sensitivity and use Gen5's Synchronized Modes in this chapter.

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Fluorescence Analysis

When Fluorescence is the chosen detection method, the **Read Step** presents the filter sets installed in the reader or defined in the Filter Wheels Library. When you're running a Synergy 2, or a Synergy HT or FLx800 with Injectors, you can perform fluorescence analysis in [Synchronized Mode](#).

Read Type options for fluorescence analysis (depending on the reader) are:

- Endpoint
- Area Scan
- Time Resolved (TRF or TR-FRET)
- Polarization (FP)

Read Speed is a **Synergy 2** option: Click the 3-dot button to change the default settings for [Measurement Options](#)

 The radio buttons (up to 6) are used to determine the number of [Filter Sets](#) to be used for the read. Only two filter sets are permitted if the reading is within a Kinetic loop. Some applications call for multiple filter sets. **Excitation** and **Emission Filters** can be selected using the drop-down list of filters available for the current reader. The list is defined from one or two sources: the [Filter Wheels Library](#) or the [Reader Setup](#)

[Filter Switching](#) is offered when only two filter sets are defined (described below). It is similar to Synchronized Well Mode.

[Optics Position](#) can be set to Top or Bottom for each selected filter set, depending on the reader's capability. Readers are equipped with one or two optical probes, positioned above (Top) and/or below (Bottom) the assay plate. **Synergy 2** offers mirror selection for the Top probe.

[Sensitivity](#) of the PMT can be set by entering the desired value (valid settings **range between 25-255**) or by letting Gen5 determine the optimal sensitivity setting using the Filter Set **Options** (described below)

Sensitivity Guidelines:

Detection	Low	High
Fluorescence: Synergy HT and FLx800	40	120
Fluorescence: Synergy 2	35	120
Luminescence	100	160
Time-Resolved FL: Synergy HT	150	255
Time-Resolved FL: Synergy 2	100	160
Fluorescence Polarization	35	120



Filter Set Options: Each filter set has a corresponding set of measurement options which can be defined by clicking the Options button. See page 148.

Filter Switching: When two filter sets are selected Gen5 offers this option to read every well with each filter set before moving onto read the next well. When this option is not selected the reader reads the whole plate with one filter set, then reads it again with the second filter set.

The advantage of this option is closely timed well measurements. The disadvantage is a longer runtime. It is useful when working with two, possibly unstable, fluorescent labels. Learn more in Gen5's Help.

Top Probe Vertical Offset: parameter is available when at least one of the filter sets uses the "top" optics position. It allows you to define how far the top probe is positioned from the top surface of the plate during the read. The valid range is determined on a plate-by-plate basis. The calculation is based on the plate height and the maximum travel of the top probe. Learn more in Gen5's Help.

Troubleshooting:

If you're not getting expected results review these suggestions:
[Troubleshooting Fluorescence/Luminescence Measurements](#) on page 161.

Luminescence Analysis

When Luminescence is the chosen detection method, the **Read Step** presents the emission filters for the current reader or you can select Hole for no filtering. The excitation filters are not available for selection, because the reader will automatically use a Plug in the excitation filter wheel. When you're running a Synergy 2, or a Synergy HT or FLx800 with Injectors, you can perform luminescence analysis in **Synchronized Mode**. Also see **Luminescence Best Practices** in Gen5's Help.

Read Type for luminescence analysis is limited to **Endpoint**

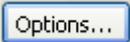
Integration Time is set as minutes:seconds.milliseconds (MM:SS.ss).

- **Synergy HT and FLx800:** Gen5 reports the measurement results as RLU (Relative Luminescence Units) per Second, except in Well Mode Kinetic experiments, which are reported as RLU, and integration time is replaced with the kinetic timelines. Gen5 collects data in each well for the duration of the integration time, then sums the data points and displays the result as RLU/second.
- **Synergy 2:** Gen5 reports the measurement results as RLU (Relative Luminescence Units). The reader collects data in each well for the duration of the integration time, then averages the data points and displays the result as RLU.

 **You should know:** in Luminescence Reading Mode there is a tight link between CV's and integration time. This correlation is independent of the instrument (type, brand) and of the reagents. If you increase your measurement time by a factor of 2, you will decrease your CV's by the square root of 2. If you increase your measurement time by a factor of X, you will decrease your CV's by the square root of X.

Filter Sets

-  The radio buttons (up to 6) are used to determine the number of **Filter Sets** to be used for the read. Only two filter sets are permitted if the reading is within a Kinetic loop. There are numerous applications calling for multiple filter sets. **Emission Filters** can be selected using the drop down list of filters available for the current reader.
- The recommended **Optics Position** for Luminescence readings is **Top**.
- **Sensitivity** of the PMT can be set by entering the desired value (valid settings **range between 25-255**), or by letting Gen5 determine the optimal sensitivity setting using the Measurement Options (learn more on page 150)
- **Sensitivity Guidelines:** BioTek recommends a setting between **100-160** for **Luminescence**

-  **Filter Set Options** - For each filter set you can use Gen5's tool to automatically determine the optimal Sensitivity of the PMT. See page 148.
- **Top Probe Vertical Offset** parameter is available when at least one of the filter sets uses the "top" optics position. Generally, you do not have to change the default value. For all Luminescence assays Gen5 sets the probe as close to the well as possible.

Time-Resolved Fluorescence Analysis

The Time-Resolved Fluorescence option is available if the selected detection method is Fluorescence and the current reader supports it.

1. For each **Excitation** filter set:
 - **Synergy HTRR**: enter a wavelength value between 200 and 999 nm. The bandpass is not variable; it is pre-defined to be 10 nm.
 - **Synergy 2**: select the filter, as usual. The Light Source is set and fixed to use the Xenon Flash.
2. For each **Emission** filter set, use the drop-down lists to select the wavelengths.
3. **Sensitivity** can be set by entering the desired value (**the allowable range is 25-255**), or by letting Gen5 determine the optimal sensitivity setting using the Filter Set Options (learn more on page 150). **Sensitivity Guidelines**: BioTek recommends a setting between **150-200** for **Time Resolved Fluorescence**
4.  click the **Options** button when using the **Synergy HTRR** or click the 3-dot button next to the Time Resolved checkbox when using the **Synergy 2**: Specify the length of time to delay before data collection and the data collection duration and define the other related measurement options. For the Synergy 2 they are offered as Read Speed settings.

FYI:

The measurement of fluorescence of compounds using a delay after the cessation of excitatory light is referred to as time-resolved fluorescence (TRF). In many biological systems, auto fluorescence, within the sample or the microplate, is a common source of background fluorescence. Lanthanide ions, particularly europium and terbium, have extremely long fluorescent decay times, measured in several hundred microseconds, whereas typical fluorescence is measured in several nanoseconds. The rapid on/off nature of a xenon flash lamp allows for the analysis of the fluorescence of these compounds with a delay after the excitation flash. The subsequent delay allows for virtually all of the background fluorescence to diminish prior to measurement of lanthanide's long-lived fluorescence, resulting in superior detection limits.

Fluorescence Polarization

For each Fluorescence Polarization (FP) Read step, Gen5 actually performs two reads. It takes a measurement through a parallel polarizer, and then, through a perpendicular polarizer. Two raw data sets are generated. Gen5 automatically transforms the data in an FP-dedicated data reduction step.

When applicable, Gen5 also performs the other automated data reductions, blank subtraction and kinetic well analysis on the FP data sets. Anisotropy is also offered as a data reduction option for FP analysis. You can choose to transform the data to produce Polarization results, Anisotropy results or both. Results are reported as **mP** (millipees), with an expected range between 0 - 500 mP with precision of ± 2 mP.

When the Synergy 2 is equipped with the polarizers and mirrors required to excite the sample and capture the polarized emission, the polarizing lenses reside in position 3 of the mirror holder. Thus, when FP is the selected read type, the Optics Position choice of mirrors is fixed to position 3.

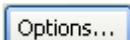
Top 5 Things to Know about Fluorescence Polarization

- Mirror selection is fixed by Gen5 to use position 3. If you have a dichroic mirror in position 3 (which is the default configuration), you must select filters that correspond to its Min-Max range
- When Gen5's Auto-Sensitivity adjustment is used to determine the optimal PMT sensitivity, it is performed on the parallel read/measurement
- Either light source can be used for FP: **Tungsten** or **Xenon Flash**
- **Read Speed** can be set to sweep when the Xenon Flash is used
- **Synchronized Well** and **Plate Modes** are available for time-sensitive FP assays

Synergy 2 FP Process

For each sample in an FP experiment, the Excitation light travels from the light source through the EX polarizer and then is reflected by the mirror to excite the sample. The sample's emitted light travels through the mirror and parallel Emission (EM) polarizer and then through the emission filter into the PMT. The mirror holder then shifts so that the emitted light travels through the perpendicular EM polarizer, through the emission filter, and into the PMT. Gen5 corrects for the optical variations between the parallel and perpendicular emission paths during the FP data reduction step. Learn more in the Data Reduction chapter, page 297.

Filter Set Options



In Fluorescence and Luminescence assays, you can set the sensitivity level of the PMT or let Gen5 determine the optimal setting. You may need to experiment with the settings to find the combination of options that works best for your assay. In Fluorescence analysis, you can also set the Measurement Options.

Automatic Sensitivity Adjustment

Click in the checkbox to use this option to determine the optimum **Sensitivity** setting for the plate:

1. First, select one:
 - **Scale to High Wells** - to evaluate optimal sensitivity based on the strongest signal
 - **Scale to Low Wells** - to evaluate optimal sensitivity based on the weakest signal
 - **Use First Filter Set Sensitivity** of This or the First read step - available when there is more than one filter set and/or more than one read step of the same detection method
2. Then, define:
 - **Scale Wells** - a range of microplate wells with the highest or lowest expected signals, click in the field to select one or a range of adjacent wells, e.g., A1-B12
 - **Scale Value** - the highest or lowest expected value for the entire plate.

For input recommendations review the valid value ranges in the PMT Sensitivity section on page 150.

Luminescence

Gen5 performs the Automatic Sensitivity Adjustment based on an integration time of 1 second (0:01:0) regardless of the Integration Time defined for the Read step, except in the Synergy 2, which uses the Read step's integration time. The Synergy 2 may be unable to determine the optimal sensitivity, especially when scaling to low wells, if the integration time is > 1 second or < 1 millisecond (0:00:01).

Fluorescence Measurement Options

The values for each measurement option depend on whether the assay is defined as **Standard** (Non-Synchronized Mode), Synchronized **Plate Mode** or **Well Mode**. This table displays the recommended values and **Allowable Range** for these modes for each option. **Note:** For Kinetic analysis in Well mode these options are unavailable, they are determined by the kinetic interval.

Option	Standard & Time Resolved	Plate Mode	Well Mode	Allowable Range
Delay after plate movement	100 msec	250 msec	10 msec	10-2550 msec
Measurements per well	10	10	1	1-255
Delay between measurements	1 msec	24 msec	0	0-255 msec

Time-Resolved Fluorescence (*except for Synergy 2*)

Delay before data collection: is time delay between the flash and the beginning of data collection or the delay before integration. Valid values are **0 µsec** and **20 - 16000 µsec**. The default value is 20 µsec.

A delay of 0 µsec is not the same as a fluorescence read with the time-resolved option turned off. They differ in two ways: (1) the excitation wavelength is generated by the monochromator; and (2) the user can specify the data collection time, which is not an available parameter for normal fluorescence reads.

Data collection duration: is the amount of time for which readings are collected after the delay before integration time has expired. The valid range is from **20 - 16000 µsec**. The default value is 100 µsec.

PMT Sensitivity

In fluorescence and luminescence assays, the signal can be very weak, very strong, or anywhere in between. For each assay, the **Sensitivity** of the photomultiplier tube (PMT) should be adjusted to ensure that the signals from all wells fall within the appropriate dynamic range of 0 to 99998 relative fluorescence or luminescence units (RFU or RLU).

Options...

Automatic Sensitivity Adjustment

You can let Gen5 determine the optimal sensitivity setting: click **Options** and provide some criteria on which a determination can be made.

i Guidelines:

- **Typical fluorescent assays** using 96- (or fewer) well plates require sensitivities between 35 and 150. Assays using 384-well or other higher-density plates will likely require higher sensitivities due to narrower optical probes. **Luminescent reactions** may require sensitivities up to 200. BioTek recommends selecting a PMT Sensitivity setting between these ranges:

Detection	Low	High
Fluorescence: Synergy HT and FLx800	40	120
Fluorescence: Synergy 2	35	120
Luminescence	100	160
Time-Resolved FL: Synergy HT	150	255
Time-Resolved FL: Synergy 2	100	160
Fluorescence Polarization	35	120

- As the sensitivity setting increases, so will the fluorescence values. If many wells result in “OVRFLW,” the sensitivity setting is probably too high:
 - OVRFLW indicates RFU or RLU values greater than 99998 for Synergy HT, FLx800 and Synergy 2 in Standard Range (e.g. with Xenon Flash)
 - OVRFLW indicates RFU or RLU values greater than 5.8 million for Synergy 2 in Extended Range.
- **Recommendation:** one way to determine the optimal Sensitivity setting is to set up one Read Step to perform 6 filter-set reads each applying a different Sensitivity. Review the results to determine the best setting.
- If the field shows ‘Auto’, the **Automatic Sensitivity Adjustment** feature is enabled to determine optimum sensitivity. See [Filter Set Options](#) above.

Automatic Sensitivity Adjustment for PMT

Gen5™ can determine the optimum PMT **Sensitivity** setting for the plate based on one of three methods: **Scale to High Wells**, **Scale to Low Wells**, or when multiple filter sets are used: **Use First Filter Set Sensitivity**. The best method depends on your application (some experimentation may be necessary).

Scale to High Wells

High Wells are wells with the highest expected signal (measurement value) on the plate, such as the **highest standard wells**.

Scale to High Wells searches for two consecutive gain values where the measured values are lower and higher than the defined high well values.

Scale to Low Wells

Low Wells are wells with the lowest expected signal (measurement value) on the plate, such as **blank wells**, **negative controls**, or the **zero standard wells**.

Scale to Low Wells searches for two consecutive gain values where the measured values are lower and higher than the defined low value wells. Scale to Low Wells is recommended for weakly luminescent reactions.

When you let Gen5 determine the optimal sensitivity setting for your assay, the valid values for Scale to High or Low Wells is dependent on the reader, detection method, and light source:

Dynamic Range	Synergy HT/FLx800		Synergy 2		
	FI	Lum	Xe	Tg	Lum
Standard	0-99,999 RFU		0-99,999 RFU	0-99,999 RFU	0-99,999 RLU
Extended		0-5,000,000 RLU/sec		0-5,800,000 RFU	0-5,800,000 RLU

BioTek recommends retaining Gen5's default values when the expected measurements are unknown.

Use First Filter Set Sensitivity of This or the First read step

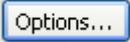
This option is available when multiple filter sets and/or multiple read steps of the same detection method have been defined. It applies the same sensitivity setting to the current filter set read.

Use first filter set from First read step: Notice that both the first filter set and first read step are referenced: it applies either the manually input or automatically determined Sensitivity setting of the first read step's first filter set. This only applies to the same detection method. In a multi-mode Procedure, Gen5 selects the first read step of the same detection method (e.g. FI or Lum).

Use first filter set from This read step is offered when there are multiple filter sets in the current read step. It applies either the manually input or automatically determined Sensitivity setting of the first filter set to the current read.

- ❖ **In Dispense protocols**, if two filter sets are defined, the Sensitivity field for the second filter set always shows Auto and it will use the first filter set's sensitivity.
- ❖ **In multiple-plate experiments**, when the Automatic Sensitivity option is used, Gen5 applies the sensitivity setting determined for the first plate, to all other plates processed in the experiment.

How to let Gen5 determine the Sensitivity setting:

1.  In the Fluorescence or Luminescence read step, click the **Options** button for the Filter Set
2. Turn on **Automatic Sensitivity Adjustment**: click the box to insert a checkmark
3. Select a button for one of the options described above:
 - **Scale Wells**: click in the field to select one or a range of adjacent wells that you expect to produce the highest or lowest measurements
 - **Scale Value**: Enter a value that represents the upper limit when scaling to High Wells and the lowest limit when scaling to Low Wells of the expected range of values for the entire microplate. The recommended high value is 50,000 to 70,000 RFU/RLU. The recommended low value is 100 to 200 RFU/RLU.

Reviewing results

After using the AutoSensitivity feature in an experiment, you can view on-screen and include in reports the applied Sensitivity value. When the read step is completed:

- to Report the Sensitivity Value in reports (see below)
- to Display the AutoSensitivity Value on-screen described on page 153

- ❖ The sensitivity value applied during the experiment is retained in the Experiment file, but not in the protocol (.prt) the experiment was based on. Unless it is updated, the protocol will continue to be defined as using the AutoSensitivity option.

Report the Sensitivity Value

Add the PMT Sensitivity values to a field group to include them in a report or export:

1. Define a fluorescence or luminescence Read Step with a sensitivity setting, either a defined value or using Gen5's AutoSensitivity determination.

2. Select **Protocol > Data Views**
3. Locate the Field Group element at the bottom of the tree, highlight **Field Group** and click **New**

❖ Another option is to select **Procedure Summary** and click **Edit**, and then modify these instructions to add the field to the existing Field Group, i.e. **Add a Row** to the group, and then start at Step 6.

4. Give the new Field Group a **Name**: e.g. Temperature
5.  Click in the first cell in the first column to enable the 3-dot button, and click the 3-dot button to open the Fields dialog
6.  Set the **Category** to **Procedure**
7. Set the **Field** to **Sensitivity** (there is one value for each read/filter set)
8. Now, you can add this field group to Reports or Export files
9. **Save** or **Save As** the Protocol to make sure this field group is available whenever you run the experiment.

Display the AutoSensitivity Value

When the Procedure is completed in an experiment:

-  Highlight **Procedure** in the menu tree and right click, select **AutoSensitivity Results**
or
- Open the **Procedure** dialog (double click the menu tree item), the sensitivity value applied during the first read step is displayed.

Measurement Options

Options...

Procedure > Read > Fluorescence > click Options



Synergy 2: Procedure > Read > click 3-dot button next to Read Speed

❖ This section describes all the possible measurement options available in Gen5. You will be offered more or fewer of them depending on your reader and the context of the Read step.

You may need to experiment with various combinations of settings to determine the optimal value for each. Generally it is a trade off between speed and precision. Increase the number of measurements per well for better precision; decrease them to increase speed.

Define the measurement parameters for read speed:

- **Delay after plate movement** is the time between the end of the movement of the plate carrier (plate is in the read position) and the beginning of the acquisition of the data. The valid range is from 10 to 2550 milliseconds. The recommended setting to ensure that the fluid has settled in a 96-well plate is 100 milliseconds. This parameter is especially important in absorbance mode, where the vibration of the liquid's surface meniscus just after a plate movement can lead to variations in OD measurements.
- **Measurements per data point** are the number of measurements the reader takes per well per read. The data point reported for each well represents the *average* of its measurements. The valid range is from 1 to 255 measurements. Usually, the more measurements per well the better the CVs, although selecting a large number of measurements typically results in only marginal improvement. Consider a setting that represents the optimal combination of precision and speed. The recommended setting to achieve a balance between speed and precision is 10 measurements per well. *This is not adjustable in Absorbance reads.*

❖ **Note:** The reader takes approximately 10 milliseconds to perform each measurement.

- **Delay between measurements** is the time delay between measurements taken in each well. The valid range is from 0 to 255 milliseconds. The recommended setting is 1 millisecond. Longer delays between samples may result in better CVs between replicates.

- **Dynamic Range:** offers two ways to express the measurements taken by the reader:
 - **Standard Range:** reports measurements between 0 - 99,999, higher values are reported as "OVRFLW." This is the only setting available for Synergy HT, FLx800, and Synergy 2 when the Xenon Flash is used, e.g. for TRF.
 - **Extended Range:** reports measurements between 0 - 5,800,000, higher values are reported as "OVRFLW."

Some of the benefits of **Extended Range**, you can:

- take measurements with a high Sensitivity setting without fear of over-ranging the wells,
- measure samples with very low and very high outputs in the same Read step,
- reduce the risk of over-ranging in kinetic mode (when the signal grows over time).

Synchronized Modes

Synergy 2, Synergy HT/FLx800 with Injectors: Protocol > Procedure > Synchronized Modes

Gen5 supports three modes of processing plates. All readers can process plates in the standard mode, we'll call **Non-Synchronized Plate Mode** to distinguish it from the other options. If you're running a Synergy 2, or a Synergy HT or FLx800 with Injectors, you have two more processing mode options: **Synchronized Plate Mode** and **Well Mode**. These options give you more control in time-sensitive procedures.

 **Tip:** When defining a **Synchronized Plate Mode** and **Well Mode** procedure, in the Procedure dialog the first step is adding the **Well** or **Plate** mode block. Then, put the Read and other steps inside the block.

Non-Synchronized Plate Mode

Non-Synchronized Plate Mode is the standard mode for processing and is supported by all BioTek readers. Each step in a Procedure is processed for the entire plate (or partial plate) before the next step is processed. No attempt is made to maintain consistent well-to-well timing from one step to the next.

Multi-Detection Kinetics

For readers that support multiple detection methods (absorbance, fluorescence, luminescence), the Non-Synchronized Plate Mode supports the ability to include multiple read steps in a single kinetic block. This capability is called Multi-Detection Kinetics.

With a Synergy HT, for example, in Non-Synchronized Plate Mode, you can perform a four-wavelength absorbance read, followed by a kinetic loop that includes a dual-wavelength fluorescence read.

Synchronized Plate Mode

In Synchronized Plate Mode each step is processed for the entire plate (or partial plate) before the next step is processed, but the time spent at each well is identical. Steps within a Synchronized Plate Mode block maintain consistent well-to-well timing, such that the time required to process two successive steps is the same for each well on the plate. The Synergy HT and FLx800 readers can only perform Fluorescence in Plate Mode; the Synergy 2 can perform all detection methods.

When timing is critical to your research, Synchronized Plate Mode gives you the ability to perform a precisely timed sequence of steps on each well. For example, you can define the timeline for a Dispense>Wait>Read block of steps that encompasses the time to complete all three steps from beginning to end, so you know precisely when reagent was dispensed to each well and when it was read.

Synchronized Well Mode

In Synchronized Well Mode, all steps within a block are performed on a single well, before proceeding to the next well. Like Synchronized Plate Mode, the time spent at each well is exactly the same. The Synergy HT and FLx800 readers can only perform Luminescence and Fluorescence in Well Mode; the Synergy 2 can perform all detection methods.

How to choose which option to use?

You may want to test the different options to see which works best for your experiments, but here are some general guidelines to help narrow the choice:

- When you expect the well signal to vary over time, select Synchronized Plate Mode or Well Mode (Synergy HT and FLx800 do not support a Shake step following reagent dispensing in Well Mode)
- Non-Synchronized Plate Mode is efficient when using a Stop solution and the well signal is stable over time
- In kinetic analysis, use Synchronized Plate Mode or Well Mode when it is important to have the same exact timing for each well
- In kinetic analysis, use Non-Synchronized Plate Mode when it is important to obtain as many data points as possible
- When conducting long-time-span kinetic reads (hours), Non-Synchronized Plate Mode is sufficient
- In Synchronized Mode you can define the duration of a Delay step to include the time it takes to perform the previous step
- Multi-Detection (multi-mode) Kinetic Reads can only be performed in Non-Synchronized Plate Mode

Processing Modes Comparison

Here's a tabular breakdown of the differences between **Non-Synchronized Plate Mode**, **Synchronized Plate Mode**, and **Well Mode**.

Process	Detection	Supported Steps	Kinetic Loop Steps	Read Settings	Partial Plate Processing
Non-Synchronized Plate Mode	Absorbance Fluorescence Luminescence	All Procedure steps supported by Reader	Shake Read	Can define unique settings for each read step	Can define unique setting for each step
Synchronized Plate Mode	Synergy 2: all methods Synergy HT & FLx800: Fluorescence	Read Dispense Shake Delay Kinetic	Shake Read	Defined in first step for all subsequent steps	Defined in first step for all subsequent steps
Well Mode	Synergy HT & FLx800: Fluorescence Luminescence Synergy 2: all methods	Read Dispense Delay Kinetic Synergy 2: Shake	Read Synergy 2: Shake	Defined in first step for all subsequent steps	Defined in first step for all subsequent steps

Synchronized Mode Performance Chart

Reader	Max # of Steps	Max # Kinetic Reads	Max Kinetic Interval (sec)	Time Increment
Synergy HT	9	999	Plate = 9999 Well = 99.99	Plate = 1 sec Well = 20 ms
FLx800	3	300	Plate = 9999 Well = 12	Plate = 1 sec Well = 20 ms
Synergy 2	20	999	Plate = 9999 Well = 99.99	Plate = 1 sec Well = 20 ms

Synchronized Plate Mode Limitations

Here is a listing of the Plate Mode limitations:

- **Synergy 2** supports all detection methods, but **Synergy HT** and **FLx800** only support Fluorescence
- All parameters set in the first read step of the block are automatically applied to subsequent read steps, with the exception of Step Label and Light Shuttering
- Partial plate settings are automatically applied to all read and dispense steps using the settings in the first step
- The only available steps in a plate mode block are: Read, Dispense, Shake, Delay, and Kinetic
- When two filter sets are used in the read step, the second filter set automatically uses the sensitivity of the first, except **Synergy 2** allows different sensitivity values for each step
- A Shake step must immediately precede a Read step or a Kinetic Start step
- The maximum duration of a plate-mode block is approximately 27 hours 47 minutes for **Synergy HT** and **FLx800** and approximately 277 hours 47 minutes for the **Synergy 2**
- The only available steps within a plate mode kinetic loop are Shake and Read and the maximum runtime duration for a loop is 24 hours
- A maximum of one read step and one shake step is supported in a plate mode kinetic block
- 384- and 1536-well plates are not supported
- In the **FLx800**, a plate mode block must contain at least one kinetic loop to be valid. If two kinetic loops are present in the plate mode block, they must have the same kinetic interval
- In the **FLx800**, a plate mode block can contain only one dispense step, and a dispense step cannot be the last step in the plate mode block
- Light Shuttering is available

Synchronized Well Mode Limitations

Here is a listing of the limitations that apply to Well Mode blocks:

- Synergy 2 supports all detection methods, but Synergy HT and FLx800 only support Fluorescence and Luminescence
- All parameters set in the first read step of the block are automatically applied to subsequent read steps, with the exception of Step Label and Light Shuttering
- Partial plate settings are automatically applied to all read and dispense steps using the settings in the first step
- The only available steps in a well mode block are: Read, Dispense, Delay, and Kinetic, except Synergy 2 also supports a Shake step before a Read or Kinetic loop
- The maximum duration of a well-mode block is approximately 16 minutes 40 seconds
- The only available step within a well mode kinetic loop is a Read step, except Synergy 2 also supports a Shake step
- When two filter sets are used in the read step, the second filter set automatically uses the sensitivity of the first, except Synergy 2 allows different sensitivity values for each step
- A maximum of one read step is supported within a well mode kinetic block
- Luminescence read steps within a well mode kinetic loop do not support the entry of an Integration Time. It is replaced by the kinetic run time
- 384- and 1536-well plates are not supported
- Light Shuttering is available
- **Important:** FLx800 readers require adding a Delay step (beginning at the start of the previous step) to the Procedure to avoid potential loss of data. You must determine the correct duration of the delays

Troubleshooting Fluorescence/Luminescence

Here's a list of potential problems, the possible cause and a remedy:

Fluorescence / Luminescence Readings Too Low

- [Possible cause: The Sensitivity in the Read Step dialog is currently set too low](#)

Raise the Sensitivity to an appropriate level:

Detection	Low	High
Fluorescence: Synergy HT and FLx800	40	120
Fluorescence: Synergy 2	35	120
Luminescence	100	160
Time-Resolved FL: Synergy HT	150	255
Time-Resolved FL: Synergy 2	100	160
Fluorescence Polarization	35	120

If using Automatic Sensitivity Adjustment, use the Scale to High Well option and set the target value to be between 20,000 and 80,000 for standard range; or 1,000,000-3,500,000 for extended range.

- [Possible cause: The wrong filters are selected in the Read Step dialog](#)
Examine the current filter settings and make any corrections. If the filter settings appear to be correct, check the locations of the actual filters in the instrument.
- [Possible cause: Top probe is too high](#)
 - **Synergy HT:** If a plate cover is not being used, lower the top probe to 1 mm above the selected plate using the [Top Probe Vertical Offset](#) option in the Read Step dialog
 - **Synergy 2:** Gen5 generally positions the top probe at the optimal height for fluorescence reads: it focuses the beam above the well. Refer to Gen5's Help and use the [Top Probe Vertical Offset](#) option in the Read Step dialog to make adjustments
 - **FLx800:** Refer to the Operator's Manual for instructions to manually lower the Top Optical Probe

Fluorescence Background Too High

- [Possible cause: Using incorrect microplates](#)

Solid black plates and top probe reading lower the background. Black plates with clear bottoms lower the background if bottom reading is necessary. Corning 3615 or 3614 (for cell culture) are appropriate.

- [Possible cause: The wrong filters are selected in the Read Step dialog](#)
Examine the current filter settings and make any corrections. If the filter settings appear to be correct, check the locations of the actual filters in the instrument.
- [Possible cause: Phenol red is used in the media when exciting at 485 nm and reading at 528-530 nm](#)
Eliminate or replace the phenol red
- [Possible cause: Cells, media and other contents fluoresce](#)
Use deionized-water blank wells as a diagnostic tool. The blank-well reading will help you determine the background value contributed by the instrument, labware and media.
- [Possible cause: The top and/or bottom probe needs cleaning](#)
Refer to the Operator's Manual for instructions to open and clean the reader's internal components.
- [Possible cause: The instrument has fluorescing material spilled inside](#)
Refer to the Operator's Manual for instructions to open and clean the reader's internal components.
- [Possible cause: The Sensitivity in the Reading parameters dialog is currently set too high](#)
Lower the Sensitivity setting. The background should still read higher than zero. 200 is a recommended minimum and a value of 1000 takes advantage of the system's 5-digit resolution.

Reader Not Achieving Desired Fluorescence Detection Limit

- [Possible cause: The wrong filters are selected in the Read Step dialog](#)
Examine the current filter settings and make any corrections. If the filter settings appear to be correct, check the locations of the actual filters in the instrument.
- [Possible cause: Using incorrect microplates](#)
Solid black plates and top probe reading lower the background. Black plates with clear bottoms lower the background if bottom reading is necessary. Corning 3615 or 3614 (for cell culture) are appropriate.
- [Possible cause: The Sensitivity is currently set too low](#)
Raise the Sensitivity setting until the background wells read at least 200 RFU, (1000 RFU is preferred) in the Read Step dialog
- [Possible cause: Readings are taken using the bottom probe](#)
Switch to the top probe (Optics Position in the Read Step dialog)
- [Possible cause: The solution volume is 50 ul or less](#)
Increase the solution volume to 150 - 200 ul, if possible.
- [Possible cause: Wrong pH](#)

Fluorescence is very pH dependent. Use the appropriate pH.

- [Possible cause: Phenol red is used in the media when exciting at 485 nm and reading at 528-530 nm](#)

Eliminate or replace the phenol red.

- [Possible cause: Top probe is too high](#)
 - **Synergy HT:** If a plate cover is not being used, lower the top probe to 1 mm above the selected plate using the [Top Probe Vertical Offset](#) option in the Read Step dialog
 - **Synergy 2:** Gen5 generally positions the top probe at the optimal height for fluorescence reads: it focuses the beam above the well. Refer to Gen5's Help and use the [Top Probe Vertical Offset](#) option in the Read Step dialog to make adjustments
 - **FLx800:** Refer to the Operator's Manual for instructions to manually lower the Top Optical Probe
- [Possible cause: Transfection efficiency in gene expression is too low](#)
Use more cells, or improve the transfection efficiency.
- [Possible cause: DNA is old or of poor quality](#)
Use high quality, new DNA.
- [Possible cause: Not using nuclease-free buffer in DNA quantitation](#)
Use nuclease-free buffer.
- [Possible cause: Poor dilution methods](#)
Use appropriate dilution method in tubes.

Reader Over-ranging in Fluorescence

- [Possible cause: The Sensitivity in the Read Step dialog is currently set too high](#)
Lower the Sensitivity setting. Refer to the **Sensitivity** table on page 161 for valid values.

If using Automatic Sensitivity Adjustment, try the Scale to High Well option and set the High Value in the range of 50,000 to 70,000 for standard range.

Bandwidth Verification Failed

- [Error or warning messages are issued when Gen5 detects overlapping wavelengths or bandwidth](#)
Select/enter [Filter Set](#) wavelengths that do not overlap. Learn more about Gen5's bandwidth verification in Gen5's Help.

Error during Auto-Sensitivity Determination

- [Reader cannot fulfill request to determine optimal Sensitivity](#)
Gen5 displays an error message when the reader cannot determine the optimal sensitivity based on the defined reading parameters.

- **Luminescence** integration time in the Synergy 2 should be ≤ 1 sec and > 1 ms, especially when scaling to low wells.
- Manually enter a Sensitivity value or use an alternative method to determine the optimal sensitivity, if the error persists. Learn more in Gen5's Help.

Filters and Mirrors

Changing Filter Wheels

Important! It is essential to conform to the specific BioTek instrument procedures for altering the filter wheel configuration. The reader does not automatically detect which filters are installed. Before the instrument is shipped from the factory, BioTek updates the reader's internal software with the current filter wheel settings. When you make changes to a filter wheel, it is your responsibility to ensure that Gen5's wavelength table exactly matches the new contents of each filter wheel. For specific instructions on changing filters, see the reader's operator's manual. In order to obtain accurate results and prevent damage to the PMT, they must match exactly.

- ❖ To exchange wavelength information between Gen5 and the instrument, the two must be communicating: the reader must be turned on and properly configured in Gen5.

In Gen5, there are two ways to update the reader with filter wheel configuration:

- use the **Fluorescence/Luminescence** tab of the Reader Setup (System>Reader Configuration>View/Modify>Setup) to ensure the wavelengths table matches the filters installed in the attached reader
- use the **Filter Wheels Library** (System>Optics Library>Filter Wheels) to select a pre-defined wheel and send its values to the reader.

Filter Wheels Library

System> Filter Wheels

- ❖ Only for Fluorescence- and Luminescence-equipped readers
- ❖ Refer to the reader's Operator's Manual for instructions for physically changing the filter wheel

About the Filter Wheels Library

Gen5 provides these controls to create a catalog of your Fluorescence and Luminescence excitation and emission filter wheels. If you regularly change filter wheels, this feature provides significant time savings: define the filters in each wheel once, and with one click, update the reader's internal software to match the configuration of the selected wheel.

When you add new filters to the catalog, Gen5 offers them for selection when you're defining a Read step. This feature lets you create protocols, i.e. define reading parameters, using filters that are not currently installed in the reader. The **Read Plate Prompt Option** will ensure that users are alerted when they try to run a Procedure that calls for filters not currently installed.

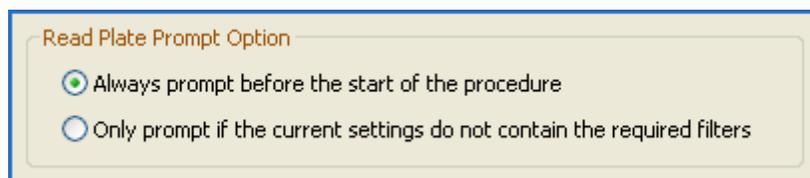
Gen5 considers filter wheels to have distinct uses, they are either **Excitation** or **Emission** wheels. BioTek ships wheels labeled either **EX** for excitation or **EM** for emission. The Time-Resolved Fluorescence, **TR** cartridge for the Synergy HTTR is NOT eligible for this catalog.

Some Read-step parameters, e.g. Light Shuttering, require a special filter-wheel configuration. This feature provides a short-cut for setting up this type of experiment. After creating a record for the special wheel in the library, you can simply exchange the wheels and with one click, update the reader's settings.

How to use the Filter Wheels Library

Select **System>Filter Wheels** to perform these functions:

1. First, the library records (descriptions of your filter wheels), must be created (page 167)
2.  When records have been added to the library, select the **Type** of wheel you want to view or modify using the drop-down list. The list box shows all previously defined wheels of that type.
3. Highlight a record and click **View/Modify** to see or change the details of a record or **Delete**, as needed.



4. Select a Read Plate Prompt Option:
 - Select the **Always prompt** option to tell Gen5 to always open the Filter Wheel Selection dialog whenever the Procedure is Validated and when a plate is read
 - Select the **Only prompt** option to tell Gen5 to only open the dialog when the Read step calls for a filter that is not in a currently installed wheel
5.  After you have physically exchanged a filter wheel, when you want to update the reader's internal software to match a selected wheel, click **Set Reader**. This performs the same function as the Reader Setting's Setup option.

 Updating the reader with a filter-wheel configuration from the Filter Wheel Library also updates the **Reader Configuration> Reader Settings> Fluorescence/Luminescence Filters** table

Creating and Modifying Filter Wheels

System > Filter Wheels > Add or View/Modify

Creating new filter wheel records

To add a new filter wheel to your library:

1. Select **System > Filter Wheels**
2. Click **Add...**
3. Select the Wheel Type: **Excitation** or **Emission**
4. For each numbered Filter position, enter the wavelength values in the **Center** fields, or use the drop-down list to select **PLUG** or **HOLE**. The wavelength and bandwidth are printed on the side of each filter.
5. For each **Center** wavelength value, enter its accompanying **Bandwidth**. This is the filter bandpass width, in nanometers, of the installed filter. For example, the Center/Bandwidth combination of 485/20 will transmit light from 475 to 495 nm (10 nm on either side of the center).
6. When the filter wheel is fully defined, click **OK**.

Special filter position requirements for the Synchronized Modes:

For Dispensing Protocols and certain other procedures, Gen5 offers an option: **Close Light Shutter**, to block the light between measurements to inhibit photo bleaching:

- Single filter-set protocols: If light blocking is enabled, a blocking filter (Plug) must be placed in the excitation filter wheel in one of the two positions next to the excitation filter that is specified in the protocol.
- Dual filter-set protocols: If light blocking is enabled, two blocking filters (Plugs) must be placed next to each other in the excitation filter wheel, except for Synergy 2; it only needs one plug.

❖ Gen5 issues an error message when the configuration of the filter wheel does not match the requirements of the Procedure/Read step.

Mirrors and Mirror Holders

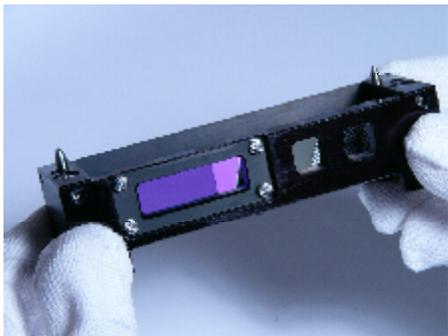
Before shipping the reader, BioTek configures the reader's internal basecode to match the mirrors installed in the reader. Unless you are changing the current configuration do not alter the settings.

Your reader's **Operator's Manual** provides detailed instructions for maintaining and changing mirrors and mirror holders.

About Mirrors

For all top-reading Fluorescence analysis, Fluorescence Intensity (FI), Fluorescence Polarization (FP), and Time-Resolved Fluorescence (TRF), the Synergy 2 uses mirrors

to direct light to the sample and obtain measurements from it. Up to three mirrors, either 50%, Dichroic, or custom, can be installed in the reader.



Mirrors reside in a mirror holder with three possible reading positions. Any mirror can be used for any type of experiment, except FP (Fluorescence Polarization), which requires using the mirror in position 3, because it holds the polarizing lenses. Keep in mind when using [dichroic mirrors](#) that the filter sets must match the wavelength range.

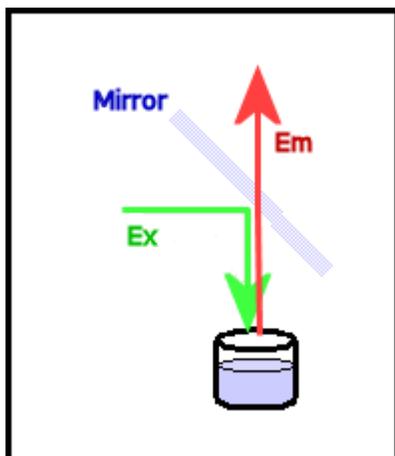
The mirror holder (pictured above) is a rectangular box located inside the reader. (Additional mirror holders can be purchased as an accessory and kept track of in the Mirror Holder Library.) The mirror holder and the mirrors are user-changeable. You can replace the entire holder with a different one; this is the recommended option. Or, alternatively, you can install different mirrors in the mirror holder. Contact BioTek for more information on purchasing additional mirrors and holders. Refer to the reader's Operator's Manual for replacement instructions.

The reader cannot detect which mirrors are installed in it. Whenever you change a mirror holder or the mirrors in the holder, use Gen5 to update the reader's internal basecode to match the new hardware.

Gen5 provides two ways to download information about your mirrors to the reader:

- Update the [Mirror Configuration](#) table when you have only one mirror holder
- or use the [Mirror Holder Library](#) when you have multiple holders

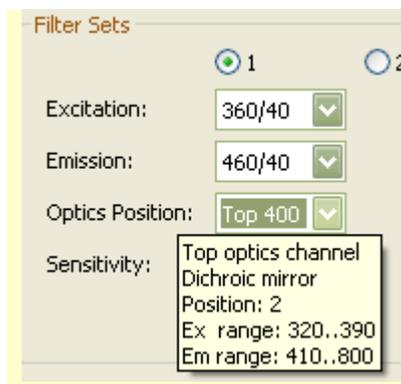
Dichroic Mirrors



Dichroic mirrors have to match the Excitation and Emission filters. If the excitation filter and dichroic do not match, the excitation light goes through the mirror, instead of reflected to the sample and the sample does not get excited. If the emission filter and dichroic don't match, even when the excitation light is reflected to excite the sample, the emission light is also reflected by the mirror and goes back to the light source instead of the detector (PMT).

Mirrors and filters are defined under Reader Configuration and then, offered for selection when the Procedure is being defined. Filters can also be defined in the [Filter Wheel Library](#).

When defining and performing a Read step, the filter set must be compatible with the mirror. 50% mirrors do not have wavelength requirements; they can be used with any filter sets. But Dichroic mirrors require the filter's [central wavelength](#) to fall within the mirror's EX and EM Min-Max range.



In the Read Step dialog, hover your mouse over the **Optics Position** to see a tool-tip that lists the Excitation and Emission range for the selected dichroic mirror. Either select filters that fall within the Ex and Em ranges (respectively) or change the selected mirror.

Mirrors Configuration

System > Reader Configuration > View/Modify button > Setup button

Normally, these controls are only needed when you are **changing a mirror or mirror holder**

To change the current settings and download them to the instrument:

When you exchange a mirror or mirror holder, follow these steps to update the reader:

1. Select **System > Reader Configuration**
2. Double-click the reader to be updated. This opens the **Reader Settings** dialog.
3. Click  **Setup...**
4. Select the **Mirrors** tab
5. For each Mirror position, **1, 2, 3**, use the drop-down list to select the **Type** of mirror. Gen5 assigns a **Label** based on your selection. For details about the valid values see [Define a Mirror Holder](#) on page .
6. For a **Dichroic** mirror, you must enter its wavelength range. Refer to the fact sheet provided with the mirror to enter the values for the **Excitation Minimum** and **Maximum** and the **Emission Minimum** and **Maximum**. Gen5 creates the **Label** for the mirror based on your input.
7. **with Polarizers**: if the mirror holder is equipped with polarizing lenses, enable this option in Gen5.
8. When the actual mirrors in the mirror holder are described correctly in the table, click **Send Values** to download the data to the reader.

Define a Mirror Holder

Gen5 provides two ways to manage information about your mirror holders:

- Use the [Mirror Holder Library](#) when you have multiple holders
- Otherwise, you can simply update the [Mirrors](#) table

To define the mirrors in a Mirror Holder:

1. Either update the library or the mirror table:
 - Select **System > Optics Library > Mirror Holders > Add or Modify**
 - Select **System > Reader Configuration > Reader Settings > Setup > Select the Mirrors** tab
2. For each Mirror position, **1, 2, 3**, use the drop-down list to select the **Type** of mirror. Gen5 assigns a **Label** based on your selection:
 - **50%** = works with any wavelength. It is a glass slide with small, reflective silver dots; 50% of the surface reflects light, 50% of the surface transmits light. It's Label is **Top 50%**.
 - **Dichroic** = works with a specific wavelength range; they are transparent to one part of the spectrum and block the other part. You must know the mirror's specific excitation and emission wavelength range to properly configure the reader.

- **Custom** = select this option when your mirror is neither a 50% nor a dichroic. Gen5 lets you select any combination of filters when defining a Read step using a custom mirror. Assign it a **Custom Name** to distinguish it from other mirrors. You can use up to 8 characters.
 - **None** = select this option when there is an empty position in the mirror holder. It is not given a label and this position cannot be selected when defining a Read step.
3. For a **Dichroic** mirror, you must enter its wavelength range. Refer to the fact sheet provided with the mirror to enter the values for the **Excitation Minimum** and **Maximum** and the **Emission Minimum** and **Maximum**. Gen5 creates the **Label** for the mirror based on your input. **Learn more...**
 4. **with Polarizers**: if the mirror holder is equipped with polarizing lenses, enable this option in Gen5.

Mirror Labels

Gen5 assigns each mirror a Label as you define or modify them. Mirrors are only used when top reading (rather than bottom reading) is performed on the plate, thus all labels (except Custom) begin with **Top**:

- 50% mirrors are always labeled **Top 50%**.
- For [dichroic mirrors](#) Gen5 calculates the average of the **Excitation Max** and **Emission Min** and assigns this value as the label. For example, Top 400 becomes the label when the EX Max = 390 and the EM Min = 410.
- Labels for custom mirrors are the user-defined Custom Name.

Mirror Holder Library

System > Optics Library > Mirror Holders...

❖ Refer to the reader's Operator's Manual for instructions for physically changing the mirror holder

About the Mirror Holder Library

Gen5 provides these controls to create a catalog of your Mirror Holders. If you regularly change mirror holders, this feature provides significant time savings: define the mirrors in each holder once, and with one click, update the reader's internal software to match the configuration of the installed holder.

First you must create a library record for each mirror holder. Then, after you physically change a mirror holder, you can select its record and instantly update the reader's internal mirror table.

When you add new mirrors to the catalog, Gen5 offers them for selection when you're defining a Read step. This feature lets you create protocols, i.e. define reading parameters, using mirrors that are not currently installed in the reader. The **Read Plate Prompt Option**

will ensure that users are alerted when they try to run a Procedure that calls for mirrors not currently installed.

❖ **Changing mirrors in a mirror holder:** the library is intended to keep track of multiple mirror holders. When you are limited to changing individual mirrors in a single mirror holder, it is quicker and easier to simply update the reader's internal mirror table using the [Reader Setup](#) controls.

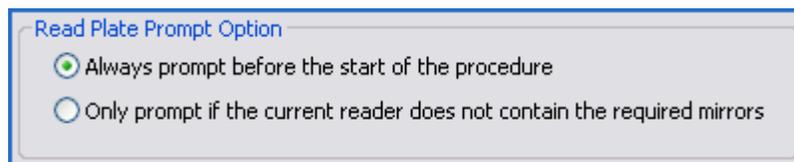
How to use the Mirror Holder Library

Select **System>Optics Library>Mirror Holders** to perform these functions:

1. First, the library records (descriptions of your mirror holders) must be created. See Define a Mirror Holder on page 170.

When records have been added to the library the list box displays them.

2. Highlight a record and click **View/Modify** to review or change its details or to **Delete** it, as needed.



3. Select a Read Plate Prompt Option:
 - Select the **Always prompt** option to tell Gen5 to always open the [Filter Wheel Selection](#) dialog whenever the Procedure is validated and when a plate is read
 - Select the **Only prompt** option to tell Gen5 to only open the dialog when the Read step calls for a filter that is not in a currently installed wheel
4.  When you want to update the reader's internal software to match a selected mirror holder, click **Set Reader**. This performs the same function as the Reader Setting's [Setup option](#).

Select a Mirror Holder

System> Optics Library> Mirror Holders...

These controls are presented during [Procedure Validation](#) and [plate processing](#) when Gen5 is instructed to "Always prompt before a procedure" or when the Read step calls for mirrors that are not currently installed in the reader.

When validating a Procedure

Gen5 opens the **Mirror Holder Selection** dialog when validating a Procedure to offer any mirror holder that meets the requirements of the read step:

1. Highlight a holder in the **Available Mirror Holders** box

2. Click **Continue** to validate the Procedure using the selected filter wheel.

When executing a Procedure

Gen5 displays an error message and, if a valid mirror holder is available, opens the **Mirror Holder Selection** dialog when the Procedure calls for a mirror that is not currently installed in the reader. Only holders that meet the requirements of the read step are offered for selection in this scenario.

3. Highlight a holder in the **Available Mirror Holders** box
4. Click **Update Reader** to select the mirror holder you will install.
5. Physically change the mirror holder in the reader. Follow instructions provided in the Operator's Manual. After it is changed and the reader is turned on and has completed its self test, return to Gen5 and click **Send Values**. The reader's mirror table is not updated until this final step is completed.

When using the Mirror Holder Library

 From the Mirror Holder Library, click **Set Reader...** when you want to update the reader's internal software to match the configuration of a selected holder

 Updating the reader with a holder from the Mirror Holder Library also updates the **Reader Configuration > Reader Settings > Mirrors** table

Multi-Detection/Multi-Mode Protocols



Synergy™ HT and Synergy™ 2 Only

Prerequisite



Currently only BioTek's multi-detection readers Synergy HT and Synergy 2 are capable of running multi-mode protocols.

Gen5™ lets you perform multiple detection methods on a plate within one protocol.

Examples

- **Stand-Alone Multi-Detection Protocol** - You can define independent read steps, performing each read with a different detection method. Other Procedure steps (e.g. Shake) can be included in the sequence, following the rules for all types of protocols.
- **Kinetic Multi-Detection Protocol** - You can perform a multi-detection kinetic analysis, conducting the reads within a kinetic loop, reading each plate with a different method in sequence. Reads must be done in Standard Plate Mode (not in Synchronized Mode).



Read the permissions and restrictions for conducting multi-detection within a kinetic loop on the next page and view examples on page 176.

Features and Restrictions of Kinetic Multi-Detection Protocols

Supported features:

- Up to 6 wavelengths for Absorbance reads
- Up to 2 filter sets for Fluorescence and Luminescence reads
- Time-Resolved Fluorescence (TRF)
- Automatic Sensitivity Adjustment
- All other valid combinations of Procedures can precede or follow the kinetic loop

Limitations:

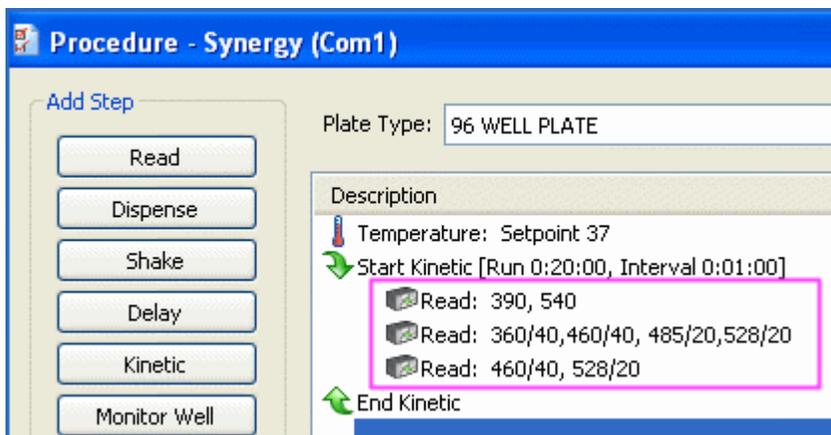
- Only Standard (non-synchronous) plate mode reads are supported
- Up to 3 read steps can be performed in a multi-detection kinetic loop
- A Shake step is the only reading-related activity that can be inside a multi-detection kinetic loop
- Synergy HT cannot perform a time-resolved fluorescence and standard fluorescence read in the same kinetic loop (nor in the same protocol). Synergy 2 can perform TRF and other types of fluorescence analysis (FI and FP) within a kinetic loop when every read within the loop uses the same [Light Source](#). TRF requires use of the Xenon Flash, so you must select this light source for any other fluorescence read within the kinetic loop
- A continuous shake event must be the first step in the sequence
- When more than one kinetic loop is defined you cannot "Append to previous Kinetic data" as is possible in a mono-detection kinetic Procedure

Features that are not supported:

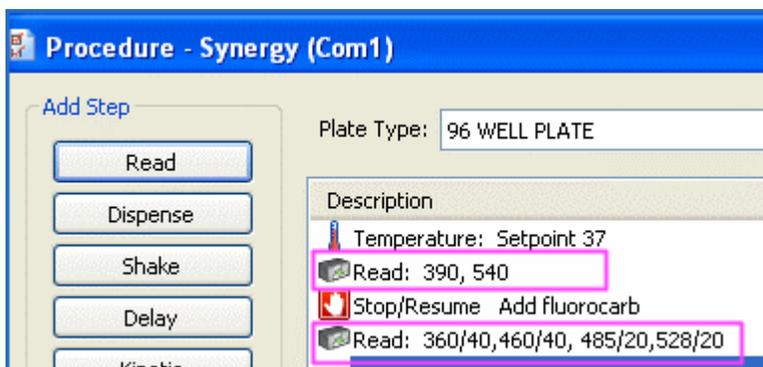
- Spectrum and Scanning read types
- Pathlength correction
- Dispense steps (Synergy 2 and Synergy HT with Injectors) (but you can work around this limitation, see [Dispensing Reagent in a Kinetic Analysis Protocol](#) in Gen5's Help)

Examples of Kinetic and Stand-Alone Multi-Detection Procedures

In a Kinetic Multi-Detection Protocol readings are performed sequentially in a kinetic loop



In a Stand-Alone Multi-Detection Protocol readings are performed independently



Chapter 9

Kinetic Analysis

This chapter focuses on the tools and techniques available for creating a kinetic analysis assay.

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Kinetic timelines	178
Kinetic Minimum Interval	180
Discontinuous (Long) Kinetic Procedure	181
Well Zoom	183
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How to set up a Kinetic Analysis

Begin with Protocol > Procedure

In the **Procedure**, for kinetic analysis, you set up the timelines and intervals to define the number of reads per wavelength and any other required activities. If you're performing time-sensitive studies review the [Synchronized Mode](#) options in the **Fluorescence and Luminescence** chapter.

To set up a kinetic analysis protocol or time course work:

1. Open **Procedures (Protocol > Procedure)**
2. Click **Kinetic**. Gen5 opens the Kinetic Step controls. Define the [timelines](#) (see the next page for more information):
 - Use the spin buttons or click in the **Run Time** and **Interval** fields and enter the desired time settings. Alternatively, let your reader determine the **Minimum Interval** for the desired Run Time. Follow these instructions for applying the **Minimum Interval on page 180**.
 - Gen5 adds a kinetic loop to the Procedure.
3. With the End Kinetic step of the kinetic loop highlighted, click **Read** to add at least one read step to the loop. If desired, include a **Shake** step before the read. (Scanning and Spectrum reads are not supported.)
4. Add other steps, including other kinetic loops, to the Procedure, as needed. Click **Validate** to make sure the current reader is capable of processing the sequence.
5. Next, go to **Data Reduction**. At least one [Well Analysis](#) calculation is automatically created for you. Double-click the Well Analysis step to view or modify it. Add more Well Analysis or other calculations, as needed.

Dispensing Reagent in a Kinetic Analysis

Select **Help > Help Topics** in Gen5 to review suggestions for including a dispense step in your Kinetic Procedure.

Kinetic timelines

Numerous factors affect the runtime parameters for a kinetic loop. The minimum interval for readings can be increased or decreased by the defined reading parameters. Here are some facts to consider and some limitations of the BioTek readers:

Reading parameters that can affect kinetic timelines:

- **Plate size:** a 96-well plate can have a shorter minimum interval and obtain more reads than a 384-well plate in the same runtime duration. Similarly, reading a partial plate generates more reads during the same time period.
- **Read Speed** in Absorbance reads: more reads can be obtained in Rapid mode than at Normal speed
- **Measurement Options** in Fluorescent reads: you can control the read speed (and affect the kinetic timelines) by adjusting the number of measurements per data point, and the delay after plate movement
- **Integration Time** in Luminescent reads: you can control the read speed (and affect the kinetic timelines) by adjusting the read duration for each well
- **Number of steps** in the kinetic loop: adding steps, a Shake and one or more Read steps, to the kinetic loop effects the timelines
- **Duration of a Shake step** in the kinetic loop is added to the Run Time

Reader limitations:

All readers are limited to obtaining 9999 reads in Absorbance mode within the 168 hour timeline. The longest interval between reads is 2.5 hours. Fluorescence and Luminescence kinetic reads and intervals are reader dependent:

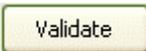
Reader	Total # Reads	Max Interval
Synergy HT: Standard mode	300	9999 sec
Synergy 2: Standard mode	9999	9999 sec
Synergy HT/Synergy 2: Synchronized Plate	999	9999 sec
Synergy HT/Synergy 2: Synchronized Well	999	99.99 sec
FLx800: Standard mode	300	9999 sec
FLx800: Synchronized Plate	300	9999 sec
FLx800: Synchronized Well	300	12.00 sec

Kinetic Minimum Interval

Here are instructions for letting Gen5 determine the shortest valid kinetic interval:

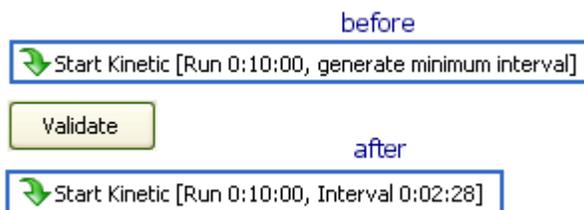
❖ **Prerequisite:** Your reader must be connected and turned on, i.e. communicating with Gen5, to determine the minimum interval

Gen5 communicates with the reader to determine how quickly the Kinetic loop can be processed. You must click the **Validate** button to trigger this communication. Follow these steps:

1. In the StepWise Procedure, click **Kinetic** to add a loop
2. Define the **Run Time** for the Kinetic loop and select **Minimum Interval**, click OK
3. Add a **Shake** step if desired and define one or more **Read** steps with the required wavelengths/filter sets and other conditions. For an Absorbance read you can increase the **Read Speed**, for faster processing. For a Fluorescence analysis, you can modify the [Filter Set Options](#) to speed up processing.
4.  Click the **Validate** button. Gen5 will display a confirmation message and the Interval will be defined.

What you'll see

You'll notice that the description of the Start Kinetic step will change from "generate minimum interval" before you hit the Validate button to a specific time setting for Interval:



❖ **Important:** Gen5 replaces the "minimum interval" setting with an actual interval time. If you subsequently make changes to the Procedure that have an effect on the kinetic interval, e.g. increase the plate size or add another read step, **the minimum interval must be recalculated.**

Discontinuous Kinetic Procedure

Procedure > Kinetic > Read

About Discontinuous Kinetics

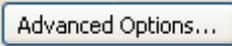
For cell growth assays and similar types of studies, Gen5 provides a way to take readings over a long time period without tying up the reader, so it can be used in other experiments during the rest or incubation periods. Gen5 calls this type of analysis: Discontinuous Kinetic, because the measurements obtained from the multiple readings are combined, resulting in a multi-read data set. There is virtually no time limit to this type of procedure.

Limitations of Discontinuous Kinetics:

- Endpoint and Kinetic reads are supported, but Area Scan, Linear Scan, and Spectral Scans are prohibited
- Synchronous Mode is not supported, i.e. you cannot perform readings in Synchronized Plate or Well Mode
- Stop/Resume steps are prohibited, i.e. you cannot include a Stop/Resume step in the Procedure

❖ Gen5's Data Reduction [Well Analysis Options](#) are available for this type of experiment

How to set up a Discontinuous Kinetic Experiment:

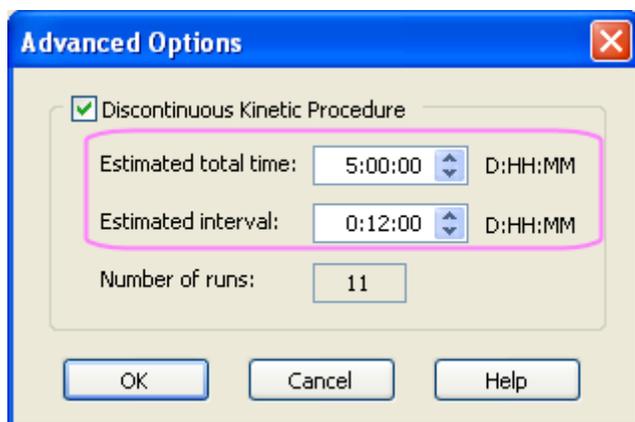
1. Create a new **Protocol** in the usual way
2.  When defining the **Procedure**, select **Advanced Options**, and select the **Discontinuous Kinetic Procedure** option
3. Enter "estimated" time points in **Days:Hours:Minutes:** for the **Estimated total time** and **Estimated interval**
This is just an estimate, and will not interfere with actual experiment activity. Gen5 uses your estimated time lines to set up the data views and formulas with placeholders until the actual data is captured. When in doubt about the required time period and intervals, it is best to **over-estimate** them. See the example on the next page.
4. Define the **Read** step(s) as usual, and save the Protocol
5. Create an Experiment based on the Protocol, and conduct the first Read on the plate
6. Close and save the Experiment
7. Remove the plate and process (e.g. add reagent) or store it (e.g. incubate), until it is time for the next reading
8. Open the Experiment, put the plate on the carrier, click the **Read** button, and select **Next Read**

- Repeat steps 5-7 until all the required reads are completed

Gen5 compiles all the reads into a kinetic data set, and performs **Data Reduction**, e.g. Well Analysis, as defined in the Protocol.

Example of Discontinuous Kinetic Estimated Time Points

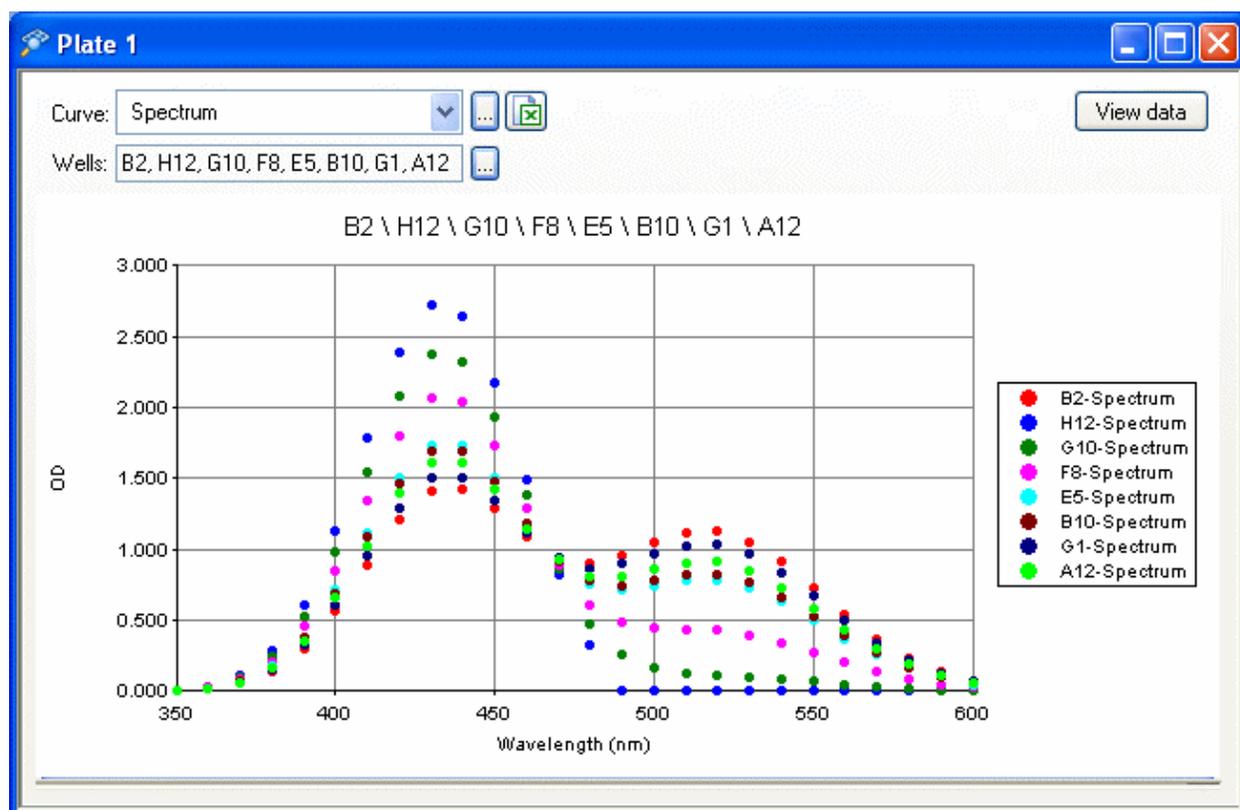
If you expect to read the plate twice a day for 3 days, but an extended time period may be needed, enter 5 days for the **Estimated total time**, and 12 hours for the **Estimated interval**:



Well Zoom

Performing a [multiple-read](#) Read Step generates the Well Zoom feature, which lets you zero-in on each well of the microplate to view the reading results for individual wells. Multiple wells can be selected for simultaneous viewing. Setting up [Well Analysis](#) as a **Data Reduction** step enhances the view, displaying the calculation results for each well.

- ❖ Gen5 automatically creates one Well Analysis step when you define a kinetic loop



How to:

Tip: To use this feature to monitor kinetic readings in real time, perform these steps **before** you start the reading. **However...see the note below.**

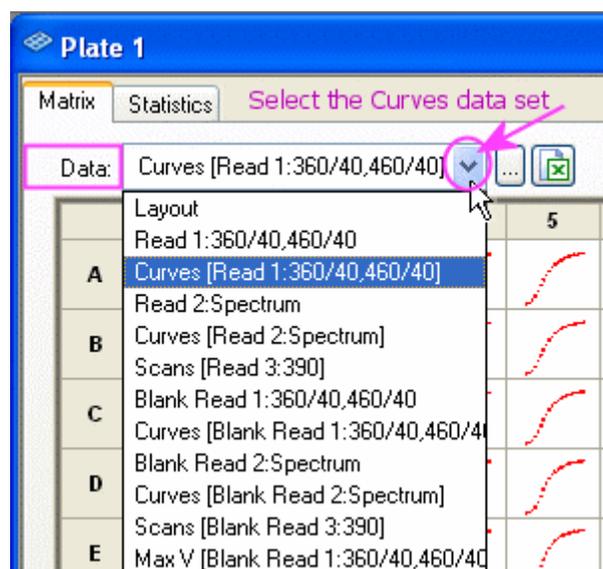
1. After reading the plate, open the Plate View that represents the plate
2. In the **Matrix** view, use the drop-down list of available **Data** sets to display the set labeled **Curves**. (The raw data set upon which a Well Analysis data reduction has been performed offers the most useful display, [for example](#): When you assign blanks to the plate, Gen5 first creates a blank-subtraction data set, and then performs well analysis on that data set. Gen5 creates a Curves data set based on

this well analysis, e.g. Curves [Blank 450] where 450 is the wavelength of the reading. Select this data set for the best well zoom.)

3. Click any well in the matrix to see its **Well Zoom** view.

❖ **Do not display Gen5's "Curves" data** in the **Plate View** while performing a kinetic analysis. Wait until the read step is finished before viewing the "Curves" data set. Displaying the **Curves** data set during a Kinetic read can consume excessive resources resulting in performance degradation. You can drill down to a **Well Zoom** to monitor the progress of one well, then, leaving the Well Zoom open, change the Matrix Data to a numeric view.

Open the Well Zoom View



Set the **Data** set in the **Matrix** view to **Curves**, and click in a well for the Well Zoom

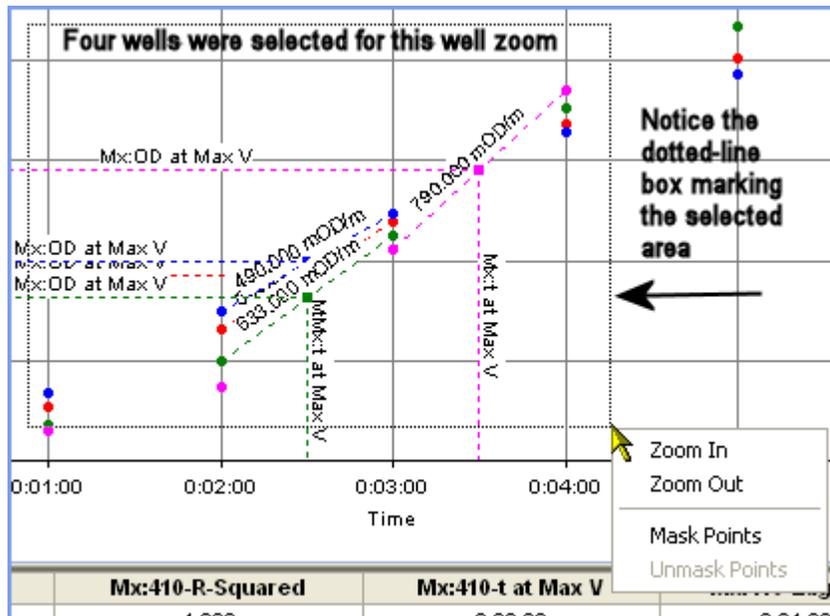
 Hold the **Ctrl** key, while selecting up to 8 wells in the Curves data set of the Plate View, to see all the well results simultaneously (except for Area Scans)

  Use the **View data**/**View chart** toggle button to show the data in either a table or graph. See examples beginning on page 190.

View Multiple Wells Simultaneously

 Click the 3-dot button next to the **Wells** field to select multiple wells to view in one graph. This is dynamic, so you can select and de-select the wells you want to see. Up to 8 wells can be selected at one time.

Zoom Zoom



Within a well zoom you can further zoom in on selected data points:

1. Click and drag your mouse over an area of the well zoom. The pointer changes to a cross as Gen5 maps the selected area. Gen5 presents a pop-up menu when you release the click
2. Select an option from the menu:
 - Zoom In or Zoom Out
 - Mask or UnMask Points

Well Zoom Plotting

When Gen5 plots the well zoom curves, the X axis represents the individual reads for the well, and the Y axis represents the measurements:

Y Axis	X Axis
ODs	Kinetic read times
RFUs	Spectrum wavelengths
RLUs	Linear horizontal positions

Well Analysis Results Table

When a Well Analysis Data Reduction step is defined, Gen5 shows the calculation results in a table beneath the curve. The "Curves" or "Scan" data set that leads to a well zoom, must be the subject of a Well Analysis step; raw data well zoom views do not include a Well Analysis Results table.

Calculation Zone

The range of reads considered for analysis is determined by the Calculation Zone setting of the Well Analysis. When the original range is reduced, Gen5 plots the revised Calculation Zone with brackets: []

Viewing Appended Kinetic Results

When one or more kinetic reads are appended, Gen5 combines them into one data set. When a Dispense step occurs between the kinetic loops, Gen5 represents the event as a blue diamond on the X-Axis timeline.

❖ **FLx800 with Injectors** may show the "Dispensing" event occurring in the same interval as a Read. This is a limitation of the reader's basecode or on-board software.

Customizing the Well Zoom View

After launching the Well Zoom dialog, you can:

- [Select a different Curve to display \(if available\)](#)

At the **Curve** field, click the  drop-down list to select another data set for the selected well

- [Mask or exclude a data point and Recalculate](#)

1. Click on a data point to temporarily exclude or mask it from the calculation, then click **Recalculate**
2. Click on the data point again to restore it

❖ **Important:** modifying data may adversely effect or possibly invalidate results!

- [Overlay up to 8 other kinetic curves on top of the current one](#)

Click the  3-dot button next to the **Wells** field to select curves from other wells to overlay onto the current one.

- [Modify or Create a new table \(to select the data displayed beneath the graph\)](#)

- ◆ Click the  3-dot button next to the **Results** field to modify the table.
- ◆ Click the  drop-down list and select **Create a New Table**
- ◆ Learn more at [Customizing Data Views, Reports, and Exports](#) in the Reporting Results chapter

- [Display or hide a legend for the graph](#)

Click  next to the **Curve** field. On the Layout tab, click in the checkbox to **Show the Legend** or remove the checkmark to hide it.

- [Modify the text and line formatting of the graph](#)

Click  next to the **Curve** field.

- [Create a new graph \(curve\)](#)

Click  at the **Curve** field and select **Create New Graph**. You can combine multiple curves, if available, with this option, to simultaneously view the well results of each curve.

Well Analysis Calculation Types

❖ Also see the **Data Reduction** chapter for useful information

Protocol > Data Reduction > Well Analysis

Gen5™ offers the following calculation types when a multiple-index read step is defined. Each option has its own specific parameters, which you define by selecting the **Calculation Options** button.

- **Mean V** is the calculated value of the mean slope. It is calculated by a linear regression on points in the **Calculation Zone**. Define the Calculation Zone with the Calculation Options button. For Mean V, the zone is typically adjusted to ignore misleading data points generated at the beginning of a kinetic assay due to "noise."
- **Max V** is the calculated value of the maximum slope:
 1. Starting at the first point in the calculation zone, Gen5 evaluates n points and calculates the slope among these n points.
 2. Gen5 repeats the operation, starting at the second point in the calculation zone, and repeats it again, starting at the third point, and so on.
 3. Finally, Gen5 compares all calculated slopes to determine the maximum slope.

Gen5 registers the **Delta t** time in the middle of the point where the **Vmax** is calculated. By default, the calculation zone starts at 2 points and at time zero.

Select the **Calculation Options** button to modify the calculation zone.

Gen5 also calculates the kinetic **Lag Time**, which is the time interval between the line of maximum slope of the propagation phase and the absorbance baseline at time = 0. Also calculated: Y Intercept, R and R², delta time at Max V, and Max V minus minimum and maximum time.

- [Mean Min/Max](#) **Mean Min** is the mean minimum OD* based on n points. **Mean Max** OD is the mean maximum OD based on n points. Gen5 calculates the Mean Min and Mean Max ODs as follows:

 - Starting at the first point in the calculation zone, Gen5 evaluates n points and calculates the mean among these n points.
 - Gen5 repeats the operation, starting at the second point in the calculation zone, and repeats it again, starting at the third point, and so on.
 - Finally, Gen5 compares all of the calculated means to determine the minimum and maximum of these values.

Gen5 registers the **Delta t** time at Min/Max OD. Select the Calculation Options button to modify the calculation zone.

*: For fluorescence and luminescence reads, Gen5 performs calculations based on fluorescence units (RFU) and luminescence units (RLU), respectively.
- [Mean, Std, CV](#) This option calculates and reports the **Mean**, **Standard Deviation**, and **Coefficient of Variation** for all points in the calculation zone. By default, the calculation zone includes all of the Reading Points defined in the protocol. To change the calculation zone, click the **Calculation Options** button.
- [Onset OD](#) Gen5™ reports:

Onset Time: the time it takes to reach **Onset OD***

Onset OD: the user-specified value. It can be defined as an absolute value or a relative value based on the **Basis OD**

Basis OD: is an optional value used to adjust all wells to a baseline. It is defined under **Calculation Options** as a fixed value or the "Mean of first n points"

Basis Time: the time it takes to reach Basis OD, when defined

*: For fluorescence and luminescence reads, Gen5 performs calculations based on fluorescence units (RFU) and luminescence units (RLU/sec), respectively.
- [Integral](#) calculates the area under the curve according to the trapezoidal method, shown here:

$$\text{Integral} = \sum_{i=1}^{i=n-1} ((y_i + y_{i+1})/2) \times (x_{i+1} - x_i)$$

Where y = measurement value and x = read point value.

The area under the curve is displayed in the Well Zoom when this option is selected.

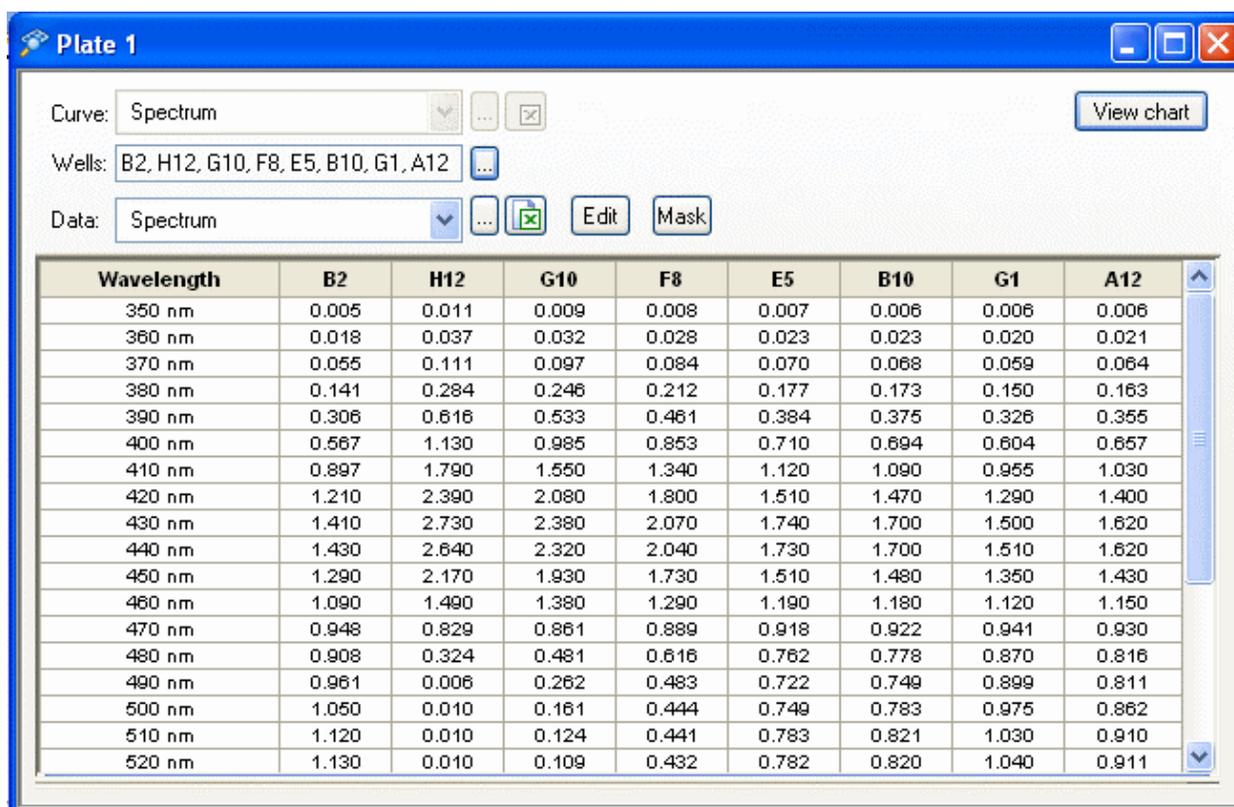
By default, the calculation zone spans the full Run Time and includes all of the reading points defined in the protocol. Click **Calculation Options** to change the calculation zone.

- [Formula](#) allows you to calculate a value using data from individual reading points. Reading points are designated R_n , where n is the reading number. Use W_n for spectrum reads.

The Formula is calculated for each well and results in one new data set named "Formula Result [nm]", where nm is the wavelength or filter set defined in the read step. When a Label is defined it precedes the naming convention: "Label: Formula Result [nm]".

For example: $(R1+R2)/2 + R10$ is read point 1 + read point 2 divided by 2 plus read point 10.

Well Zoom: View Chart/View Data



Gen5 lets you toggle the Well Zoom view between a chart and a table. Click the **View Data** button to see the table, click the **View Chart** button to view the kinetic curve.

Chapter 10

Scanning Analysis Options

Gen5, depending on the capabilities of the current reader, makes it easy to conduct an area or linear scan of wells in a plate, or a multi-wavelength spectrum scan. This chapter provides details about these capabilities.

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Area Scan

When the reader is capable of performing an area scan, Gen5 provides three ways to control the output of the captured measurements:

- **Scan Options**, defined in the Read Step of the Procedures, determine the **Read Matrix Size**. The potential Read Matrix Size is a function of the well size of the current plate.
- **Calculation Zone**, defined in the Well Analysis Data Reduction step, lets you limit the values reported, ignoring the lowest OD/RFU measurements for example, for meaningful results
- **Display Options** provide more control over the appearance of the results, letting you limit the results displayed based on their measurement values and changing the color scale applied to the values for a better presentation of the results.

❖ The Synergy 2's probe size limits its ability to perform Fluorescence area scan in plates with a small well diameter. Generally, this means you must use a plate with fewer than 96 wells.

Calculation Types

- Gen5 calculates and reports the Mean OD/RFU, Standard Deviation and Coefficient of Variation of samples

Viewing and Reporting Results

When a Well Analysis data reduction step has been defined, Gen5 displays a graphical representation of the measurements taken across the well:

1. In the **Matrix** tab of the Plate workspace, use the drop-down list of available **Data** sets to display the set labeled **Scans**
2. Click in a cell to show its **Well Zoom**
3.  Click the 3-dot button next to the **Curve** field to open the controls for adjusting the view.

In Gen5's Help you can find detailed instructions to:

- Assign a different **Title** for the graph displayed
- Hide or show the **Color Scale legend**
- Change the online view to **Gray Scale** to match the output from non-color printers
- Change the **Data Range** and **Color Range** applied to the results

Linear Scan

When the reader is capable of performing a linear scan, Gen5 plots a curve for each well using the reading points in the scan.

- **Scan Options:** You define the number of reading points in the Read Step of the Procedure

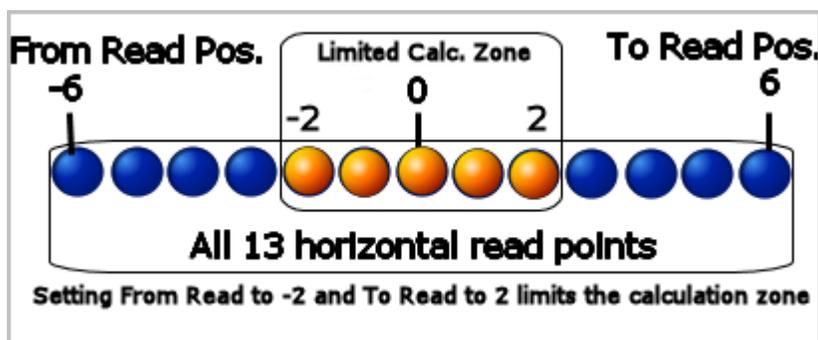
❖ PowerWave-series readers require updating the [Filter Table](#) with the wavelength you want to scan **before** reading the plate. Enter the desired wavelength in the table and click **Send Wavelengths**, then define the Read step.

Calculation Types

Gen5™ offers the following types of well analysis for linear scan:

- [Mean, Std Dev, CV](#) Gen5 calculates and reports the Mean OD, Standard Deviation and Coefficient of Variation of samples when this calculation type is selected.

You can define the **Calculation Zone**, which is based on the number of Horizontal Reading Points, which you define as **Scan Options** in the Read Step of the Procedures



- Mean Min/Max
- Integral

For each of these calculation types, you can adjust the **Calculation Zone** as desired. Click on the type to review your options and Gen5's default settings.

Viewing Results

1. After reading the plate, in the **Plate** workspace use the drop-down list for **Data** to select the data set labeled **Curves**
2. Click in a well to see the [Well Zoom](#). When a Well Analysis data reduction step has been defined, a table beneath the curve shows the results.

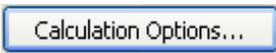
Spectrum Scan

During a **Spectrum Read**, multiple readings are taken across a wavelength range. Gen5 plots a graph with absorbance values versus wavelength. The wavelength range is defined in the Read step of the Procedure as the starting wavelength, the stop wavelength, and the wavelength interval between measurements.

Calculation Types

- Min/Max is the Well Analysis option Gen5 offers for spectrum scans. It generates the data sets: Mean Minimum OD and Wavelength at Mean Minimum OD, Mean Maximum OD and Wavelength at Mean Maximum OD

Define the Calculation Zone:



You can refine the results output of the well analysis:

- Reduce the **range of wavelengths** upon which to perform the calculations. The default zone corresponds to the **Start** (beginning) wavelength and the **Stop** (ending) wavelength defined in the Procedures.
- Reduce the **number of spectrum reads** upon which to perform the calculations. The default zone is the total number of wavelengths to be read based on the Procedure settings: from read 1 to the total number of reads calculated from the **Start**, **Stop** and **Step** values. Limiting the number of reads is another way to effectively reduce the wavelength range (nm) considered in the calculation.
- Set the **number of points** on which to average/calculate the minimum and maximum values. The default number of points is 1 (each read stands alone to determine the min and max). The valid entry range is from 1 to the total number of reads calculated from the Start, Stop and Step values.

When you increase the number of points, Gen5 first identifies the mean value for the specified number of consecutive reads, and then determines which of them is the min and max. For example, if you define 2 as the number of points, Gen5 determines the mean of the first two reads, the second two reads, the third two reads, etc., to perform the calculation. This option is most useful when there are numerous reads to work with.

Viewing the Spectrum Scan

1. After reading the plate, in the **Plate** workspace use the drop-down list for **Data** to select the data set labeled **Spectrum Curves**
2. Click in a well to see the [Well Zoom](#). When a Well Analysis data reduction step has been defined, a table beneath the curve shows the Well Analysis Results and you can use the View Data and View Chart toggle button to see the basis for the curve.
3.  Click the 3-dot button next to the Curve field to change the appearance of the well zoom.

Chapter 11

Multi-Plate Protocols

An overview and specific instructions for creating multi-plate protocols is provided in this chapter.

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About Multi-Plate Protocols.....	199
Running a Multi-Plate Protocol	201
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Designing a Multi-Plate Protocol

There are numerous applications for multi-plate protocols, not to be confused with multiple-plate experiments described on page 200.

Select the option that best meets your needs:

- **Calibration Plate Protocols**: Define a calibration or standards plate to determine concentrations of test samples on other plates
- **Multi-Plate Assay Protocol**: Use multiple plates to accommodate one assay. During processing and data reduction, Gen5 treats the multiple plates as if all samples and controls were on one plate
- **Batch Processing**: Process a batch of samples, using a limited number of standards, and incrementing continuous sample numbers across multiple plates.

❖ **Important**: All multi-plate protocols begin the same way, by first defining the number of plates required: Select **File>New Protocol** and set the **Protocol Type** as the first step.

About Multi-Plate Protocols

For the numerous applications requiring multiple-plate processing, Gen5™ helps you conduct multi-plate assays or to process multiple plates in an experiment. The method you choose depends on the requirements of your protocol. This information is intended to help you select the most efficient method.

The considerations include Plate Layout, Read Type, and data and kinetic analysis requirements:

- **Do you need more than one plate layout to distribute samples, standards, controls, and blanks?** If yes, then a multi-plate protocol is required. Otherwise, you can process multiple plates in any experiment, each containing unique Sample IDs to distinguish and track samples, but with the same plate layout (Well IDs).
- **Do you need to perform calculations on the data across multiple plates that have the same plate layout?** Multiple plates with the same layout can be processed in any experiment, but the results of each plate stand alone. Results are viewed, reported, exported and transformed individually per plate. If your assay requires determining means, ratios, or other factors across the multiple plates being processed, then you need a **Multi-Plate Assay Protocol**.

❖ **Note:** By default, Gen5 averages replicates when performing calculations. Blanks, controls, and any other identical Well ID that occurs across the multiple plates will be averaged in automatically-generated data sets. You can override this feature by creating your own transformations for individual plates.

- **Can you set up a standards or calibration plate that can be used to determine the concentration of samples that will be processed on a different plate?** If yes, the best option is the Calibration Plate Protocol, which lets you define (at least) two plate layouts, one for the calibration plate and one for all the other plates to be processed. The other plates can contain test samples, blanks, controls, etc. and like regular experiments, the results of each plate are viewed, reported, exported and transformed separately. Up to 10 calibrator plates are supported.
- **Does your assay require kinetic (or time course) analysis of the plate?** If so, your options are more limited. Gen5 does not support kinetic reads for Multi-Plate Assay Protocols

Step-by-Step Instructions:

- [Setting up a Calibration Plate Protocol](#) on page 204
- [Setting up a Multi-Plate Assay](#) on page 207

Multi-Plate Protocol vs. Experiment

❖ For this explanation you should already know the difference between an Experiment and a Protocol in Gen5™. If you haven't already done so, read [Experiment vs. Protocol](#) in the **Essential Concepts** chapter.



Important: This feature is limited by the level of Gen5 you're running:

- **Gen5 Reader Control** cannot run multi-plate protocols, but can process up to 1000 plates in an experiment. However, since Reader Control does not support Plate Layout or Sample IDs, the following explanation of Gen5's potential may not be applicable
- **Gen5 and Gen5 Secure** are full-featured software levels supporting both types of multi-plate protocols and up to 1000 plates in an experiment
- In an **Experiment** you can process a huge number of plates (up to 1000 plates) « use the **Add a Plate** feature ». Naturally, an experiment that processes more than one plate can be called "multiple plate." Regardless of the number of plates, running this kind of experiment means applying the protocol to each plate in the same manner, and transforming, reporting, and exporting data individually, on a per plate basis. Generally, every plate in a multiple-plate experiment has the same plate layout. But, you can distinguish one plate from another by assigning unique [Sample IDs](#) to each plate.
- A **Multi-Plate Protocol** allows the definition of up to 10 plates as distinct entities. Each plate in a multi-plate protocol may serve a different function, and/or have a different plate layout. It can be as simple as incrementing continuous sample numbers across multiple plates, or much more complex. Here are examples:
 - Multiple plates are required to run one assay - for quality control or other purposes it is necessary to distribute test samples, standards, and controls across a series of plates
 - Calibrator plates containing only the standards, not the test samples, are used to determine the concentration of samples on other plates. This allows for a one-time setup and processing of the calibrator plate(s), followed by the processing of up to 1000 test-sample plates in an Experiment
 - Processing a batch of samples spread over 10 plates, with incremented or user-defined sample numbers or IDs

Running a Multi-Plate Protocol

When you run a multi-plate protocol in an experiment (File>New Experiment), the menu tree, data views and reports are slightly different than a standard experiment.

Calibrator-Plate Protocols:

- Calibrator-Plate experiments add an additional set of **Protocol** elements to the menu tree for each calibrator plate and one set for all the **Other Plates**. In an Experiment, Gen5 sets up the standard Plate View for every plate, one for each calibrator and test/sample plate (Other Plates).

When you **Add a Plate** to the experiment, it takes on the attributes of the **Other Plates**.



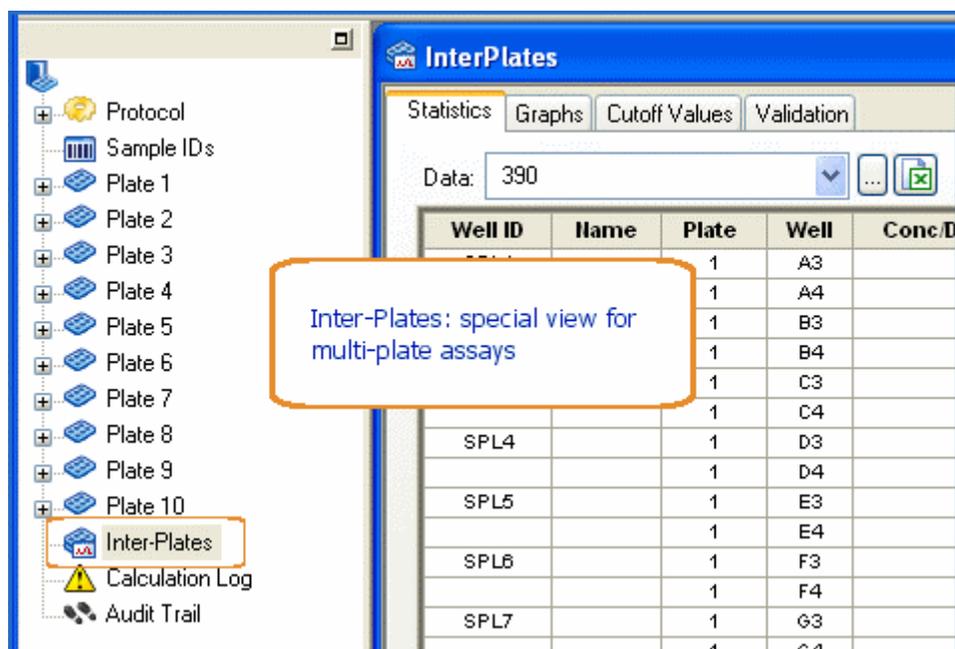
Reporting is the same as any multiple-plate experiment. After building the report, highlight a  in the menu tree and click **Print/Print Preview**. You can select multiple plates by holding the **Ctrl** key while highlighting them.

Multi-Plate Assay Protocols:

- Multi-Plate Assay experiments have one set of **Protocol** elements and a unique Data View called **InterPlates** to display the Statistics, Graphs, Cutoff Values and Validation tabs (if applicable). The Matrix for each plate is still displayed in the standard Plate View for viewing results in this format.

Unlike other types of protocols, Gen5 does not let you "Add Plates" to this type of experiment. The total number of plates required to set up the assay must be defined in the first step of creating the protocol.

InterPlates

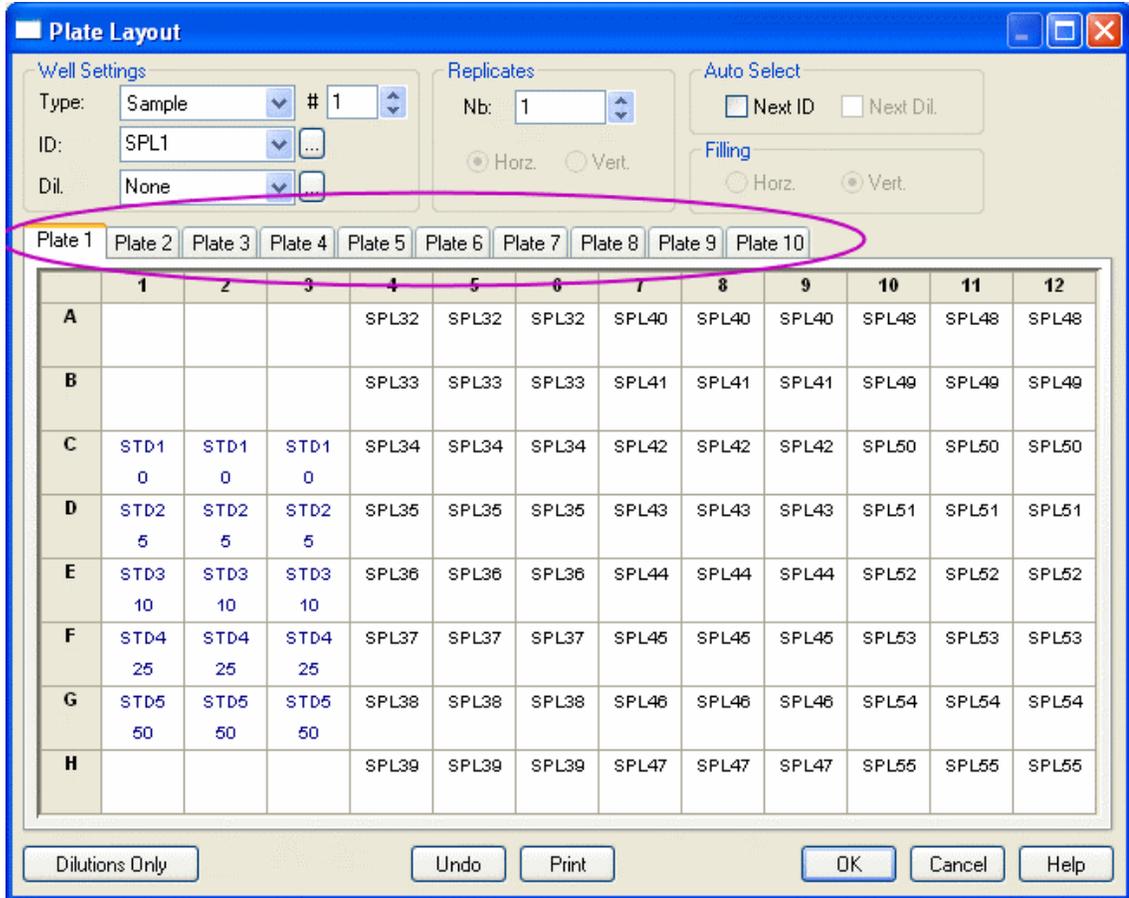


-  Reporting results for a multi-plate assay is different from standard experiments in a couple of ways. When you want to include the Matrix view in a report or export, each Plate must be added to the Content individually. And, unlike other multiple-plate experiments, all the plates are reported together in one output (instead of a separate report for each plate). Just as the **InterPlates** view (described above) displays the Statistics, curves, and other data for all the plates in one view, this data is reported/exported simultaneously for all the plates.

Plate Layout for Multi-Plate Protocols

Defining the layout of test specimens for a multi-plate protocol is identical to single-plate protocol layouts, only more so, i.e., there are more plates to define.

- Calibration plate protocols** have a unique set of protocol elements for each calibrator plate and one set of protocol elements for the test plates, called Other Plates in Gen5. So, there are additional Plate Layout options in the menu tree: one for each calibrator plate, and one for all the **Other Plates**
- Multi-Plate Assay protocols** retain one Plate Layout option but provide a grid or matrix for each plate within the dialog:



Select each Plate # tab individually to define its layout.

Data Reduction for Multi-Plate Protocols

Special formula syntax is available for performing Transformations across multiple plates:

<p><well>.<plate></p>	<p>When referencing a well coordinate in a multi-plate experiment, identify the specific well and plate using a period</p>	<p>B3.3 = well B3 on Plate 3 DS1.H6.2 = well H6 of data set 1 on Plate 2</p>
------------------------------------------	-----------------------------------------------------------------------------------------------------------------------------------	-------------------------------------------------------------------------------------------------

Review the **Formula Syntax** tables in the **Data Reduction Options** chapter for more information.

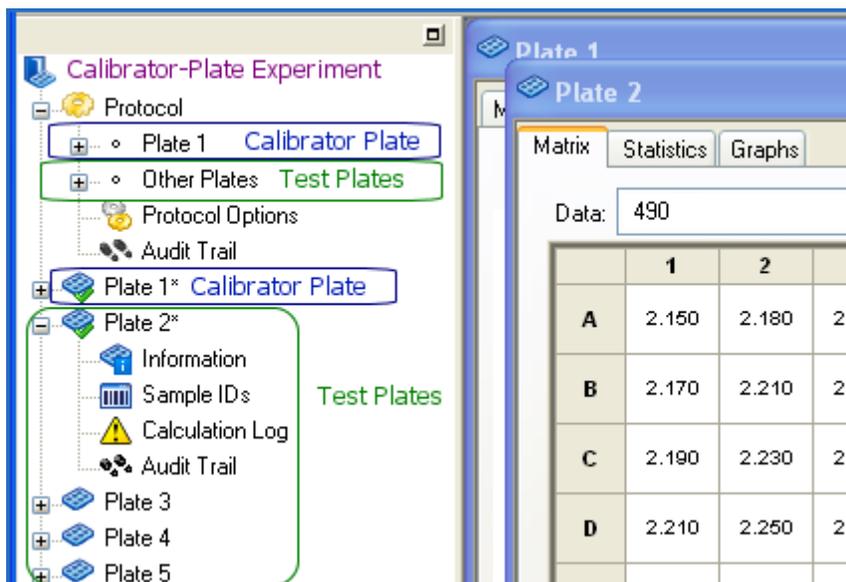
Calibrator-Plate Protocols

Creating a Calibration-Plate Protocol

This procedure defines a multi-plate protocol using a calibrator or standards plate to generate a standard curve that is used to determine the concentration of samples processed on other microplates.

Create the Protocol:

1. Begin by creating a new protocol: **File>New Protocol**
2. Select **Protocol> Protocol Options**
3. Highlight **Protocol Type** and select the **Calibration Protocol** button. Enter the number of **Calibrator** plates, maximum 10. Enter the expected number of **Other Plates**: the number of plates with samples to be processed, maximum 1000. Click **OK** to save and close the dialog



The menu tree changes to correspond to your input. Each calibrator plate is identified as "Plate #", the samples plates are identified as Other Plates. In this example (click the binoculars), **Plate 1**, the calibrator plate, is moved to the top of the tree with its own **Procedure** and other protocol elements. The **Other Plates** represent the test-sample plates and have their own Procedure, Plate Layout and so on. Generally for calibrator-plate protocols, only the Procedure for reading the plates are the same. The Plate Layout and Data Reduction steps are different. When multiple calibration plates are defined, each calibration plate has its own protocol elements.

4. Define the Plate Layout for Plate 1 and the Other Plates in the normal manner: assigning Standards with their respective concentrations to the calibrator plate (Plate 1) and assigning unknown samples with the required number of replicates to the Other Plates

5. Set up **Data Reduction** for Plate 1 and the Other Plates: define a Standard Curve for Plate 1 in the [normal way](#). Then, for the **Other Plates** define Curve Analysis using the option to **Use Curve from Calibrator Plate**
6. [Define the Report](#) output individually for **Plate 1** and the **Other Plates**. This can be a real time saver when running the protocol in an experiment

❖  Like single-plate protocols, **Calibration Protocols** allow additional plates to be added for processing when you run the experiment. Each plate is processed and its results reported separately. In Calibration Protocols, newly added plates are always **Other Plates**, rather than calibrator plates.

7. Save the protocol with a unique name.

Run the Calibration Protocol in an Experiment

When the protocol has been created, it is ready to run in an Experiment.

1. Select **File>New Experiment**.
2. Select the calibration protocol.

❖  **Plate 1**, in the menu tree, is always the first calibration plate in this type of protocol. After Plate 1, Gen5 adds one plate icon in the experiment for each calibration plate, and, one plate icon for (all) the **Other Plates**.

3. Highlight one of the plates, click **Read** and follow the online prompts. Continue this pattern, until all the plates are read.

 Each plate generates its own report (based on the user-defined report parameters). To simultaneously generate a report of all the plates, highlight the first plate in the menu tree, hold the Shift key and highlight the last plate, then click **Print**

Using Curve from Calibrator Plate

For **Calibration Protocols**, Gen5 makes available a standard curve generated from a calibration plate, to plot the concentrations of test samples on the current plate.

❖ **Prerequisite:** You must first define the Procedure, Plate Layout, and Curve Analysis for the Calibrator or standards plate. Secondly, define the Procedures and Plate Layout for the sample plate (**Other Plates**).



1. Select **Data Reduction>Curve Analysis**
2. Enter a unique **Curve Name**
3. On the **Data In** tab, select **Use Curve from Calibrator Plate** and select the previously defined calibration-plate curve
4. On the **Data Out** tab, select the **Y Data**. You can also apply **Interpolations**, if applicable
5. Also on the **Data Out** tab, enter a unique name for the **Concentration** data set. If applicable, select the **Concentration x Dilutions** calculation and give this resulting data set a unique name.

❖ Options on the Curve Fit tab are disabled (grayed out) because they are owned by the "Curve from the Other Plate."

Multi-Plate Assay Protocols

Creating a Multi-Plate Assay Protocol

This procedure creates a multi-plate protocol for an assay that requires the samples, standards and controls to be distributed to multiple plates. This method **does not support kinetic analysis**.

Create the Protocol:

1. Begin by creating a new protocol: **File> New Protocol**
2. Select **Protocol> Protocol Options**, highlight **Protocol Type** (at the top of the tree) for the multiple-plate options.
3.  Select **Multi-Plate Assay Protocol**, and enter the number of plates required to layout the assay in the **Number of Plates** field (up to 10).
4. Accept or alter the [default setting](#) for identical plate layout. Selecting **All plates have identical layout** provides only one Plate Layout grid. Leaving it unselected provides one grid for each plate.
5. Define the **Plate Layout** as you would for any protocol, using the plate-numbered tabs to bring each grid into focus. Learn more on page 202.

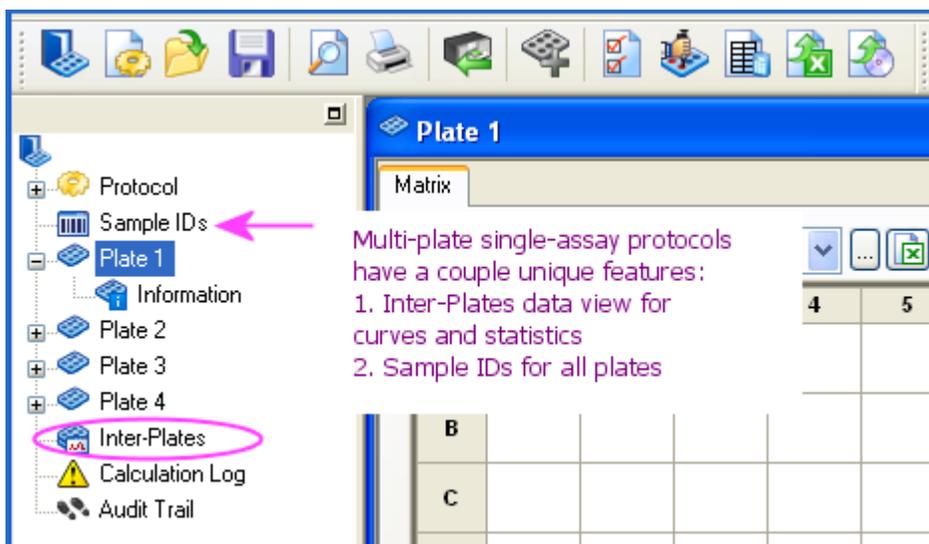
 Keep in mind Gen5's default behavior is to determine and use the mean of like-named samples in data reductions. This applies to multi-plate protocols that distribute like-named items across the plates.

6. Define the **Procedure**: Read Steps and any other necessary activities.
7. Define the **Data Reduction** steps: the calculations will be performed for all the plates at once. Gen5 will automatically create data sets for Blank subtractions if the Plate Layout and Procedures suggest them.
8. Now you're ready to define your **Report** and/or **Export** requirements.

❖ **Note:** In this type of multi-plate assay, Gen5 treats the samples from all the plates as if they were on one plate, and reports the results from all the plates together in one report. To report results in the Matrix format in reports and exports, you must add one Matrix item for each plate to the Report/Export Content. See [Designing multi-plate reports](#)

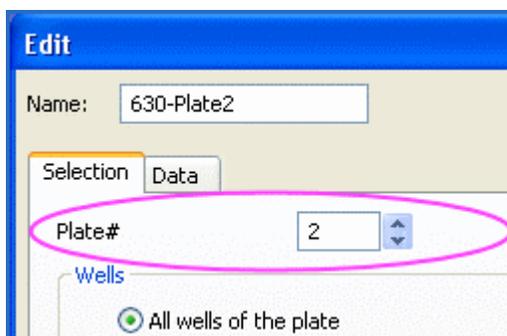
9. **Save** the protocol. And, you're ready to run it in an experiment: **File> New Experiment**.

Running a Multi-Plate Assay Experiment



If you've already [created a multi-plate single-assay protocol](#), here are a few tips for running it in an experiment.

-  **Read** each plate individually, as you would in any other experiment: highlight the plate in the menu tree and click **Read**
-  **Inter-plates** is a special view provided only for this type of protocol. Since data reductions are performed across the plates, the inter-plate view combines the statistics from all the plates into one table and shows any curves plotted from all the data points.
-  Designing the **Reports** and **Export** parameters for these kinds of assays requires extra attention: when adding **Matrix** items to the output content you must designate which plate it represents. Gen5 opens the Edit dialog to facilitate the process:
 1. Add a **Matrix** item in the Available Data Views to add it to the **Content** box.



2. Now, double-click the matrix in the **Content** box to open its **Edit** dialog. Change the **Plate number** in the **Selection** tab to identify the Plate.

3. Repeat Steps 1 and 2 until there is one Matrix item in the **Content** for each plate.

 You can fine tune the protocol while running it in an experiment, but if you like the changes, be sure to select **File>Save Protocol As** to save the changes to the original protocol for future use in another experiment.

Processing a Batch of Samples

Gen5™ offers several ways to process a large batch of samples. The method you choose depends on the requirements of your protocol.

The procedure described here uses Gen5's **Multi-Plate Assay** feature to define a protocol to process a batch of samples with one method. Gen5™ lets you assign up to 10 plates to an experiment. Plates can have the same or different layouts, but all other Protocol elements are the same.

❖ **Note:** This is one of several options for achieving the same goal. You may want to try other methods to determine which approach best meets your needs. Check out Gen5's Help to learn more.

How to:

1. Begin by creating a new protocol: **File>New Protocol**
2.  In the menu tree, open the **Protocol Options** and highlight **Protocol Type**
3. Select the button next to **Multi-Plate Assay Protocol**. Enter the number of plates required in the field, maximum 10. In this example, **each plate has a different layout** to distribute standards to some plates, and to continue sample numbering across several plates.
4. Define the **Plate Layout** for each plate. (See [Plate Layout for Multi-Plate Protocols](#) on page 202.)
5. Define the **Procedures** for reading the plates.
6. Define the **Data Reduction** steps.
7. Define the **Report** requirements.

When these steps have been completed the protocol is ready to run in an Experiment.

Chapter 12

Preparing Plates

This chapter describes Gen5's tools for defining the Plate Layout, including assigning concentration values to samples, standards, and controls, and how to customize their Well IDs. It also covers assigning Sample IDs or names to associate test samples with specific test subjects.

Defining the Plate Layout	213
Assign Standards, Controls, and Blanks	215
Customize Well IDs	215
Assign Concentrations/Dilutions	218
Customize Plate Layout.....	220
Plate Information.....	221
Runtime Prompts.....	222
Assign Sample IDs	222

Overview

Gen5™ needs to know the layout of samples, standards, controls, and blank wells on the actual microplate(s) of your experiment to perform data reduction, curve analysis, and other calculations. It is not necessary to define a plate layout to simply read the plate and report the measurements. But to take advantage of Gen5's full array of data reduction features, define the plate layout, including any concentrations or dilutions of samples, controls or standards, **before** defining the data reduction calculations.

❖ **Plate Type** for a protocol or experiment is defined in the **Procedure**. Plate Type is selected from the [Plate Types Database](#) (covered in a System Management chapter)

Gen5 supports 6-, 12-, 24-, 48-, 72-, 96-, 384- and 1536-well microplates. However, your reader may not support all of these configurations. Select the Plate Type when defining the Procedure and then open the Plate Layout to see how Gen5 formats the plate map: all columns are numbered and all rows are designated with a letter(s). Well A1 is always in the top left corner of the grid, for example:

- 96-well plates are mapped as 8 rows A-H and 12 columns 1-12
- 1536-well plates are mapped as 32 rows A-Z, AA, AB, AC, AD, AE, AF and 1-48 columns

Defining the Plate Layout

Protocol > Plate Layout

It's easy to define the plate layout with Gen5's tools for identifying samples, standards, controls and blanks. Follow these steps:

1.  In the **Well Settings** box in the top-left corner, select the **Type** of specimen
2.  Customize the **ID** or Well Identifiers, if necessary:
3.  Define the **Concentration** or **Dilution**, if applicable:
4. Assign the well IDs to their corresponding locations in the plate grid by clicking in the wells in the matrix.
 -  When you select a corresponding starting # the ID changes accordingly for assignment to the plate.
 - Use the **Auto Select** and **Replicates** options to speed up your work: set the options and click and drag to fill multiple wells at once. Click a column or row header to fill it.

❖ The type of plate, e.g., 96-well, is defined in the [Procedure](#) and displayed in a representative matrix or grid format in the Layout and Transformation screens

Helpful Hints:

- Set up your preferred default Well IDs in the [Default Protocol](#). For example, you can define PC (for Positive Control) instead of CTRL1 for Assay Controls. Well IDs defined in the Default Protocol are available when defining the Plate Layout for all newly-created protocols/experiments
- Use the **Undo** button at the bottom of the screen to undo the last action. Up to 10 previous actions can be undone
- To clear the grid and start over, right click and select **Empty Layout**, or to clear selected cells, set the **Type of Well Settings** to **Empty** and select the cells you want to clear.
- You can **Print** the plate layout. 384-well plates print out in two sections, columns 1-12 and 13-24. 1536-well plates print in eight sections to fit all 48 columns and rows from A to AF.
- To copy the contents of the grid to Windows' virtual clipboard to paste into a text/external file, right click and select **Copy Layout**. Open the receiving file, e.g. Word® or Excel® and right click and select Paste. Generally, plates larger

than 96-wells do not fit completely in a standard-sized Word or text file, a spreadsheet is required.

- Each instance of a Sample and Sample Control Well ID, and each Assay Control group can have a unique concentration/dilution value. Gen5 assigns a dilution **index** to the Well ID to keep track each instance.
- Use the **Dilutions Only/Concentrations Only** button to apply only dilution or concentration values (that you have previously defined) to selected wells without altering the well's identifier. See [Assign Concentrations or Dilutions to Samples, Standards, Controls](#) on page 218.
- Use the shortcut for filling the entire plate with the selected Well Settings.
- When assigning concentrations/dilutions, well selection must be compatible with the Replicate, Auto Select, and Filling option settings
- You can **resize** the plate view in the standard Windows® way: click and drag the outer borders of the view, or click the maximize button in the top-right corner. You can resize the rows and columns: hover your mouse over a grid line between two numbered columns or alpha-labeled rows until the cursor changes to a separator, then, click and drag.

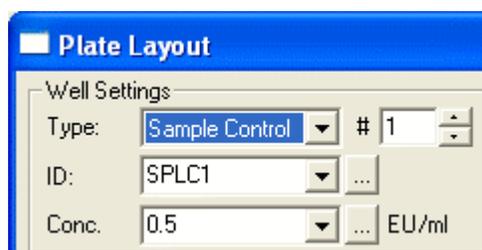
Assigning Well IDs

Protocol > Plate Layout

Gen5™ recognizes the significance of commonly-used elements in an experiment:

- **Standards** or calibrators for generating a standard/calibrator curve
- **Blanks** for blank wells subtraction
- **Controls** for multiple purposes: **Assay Control** generally used as controls, cut-offs, or for validating the assay, and **Sample Control** generally used in association with a specific sample, e.g., spiked or known-concentration samples.
- **Samples** represent the test specimen or unknowns to be processed.

When defining the Plate Layout, you can customize the Well ID and on-screen appearance, and you can assign concentration or dilution values to Standards, Controls and Samples.



Use the drop-down lists and 3-dot buttons in the upper-left corner of the Plate Layout dialog to select, customize and assign standards, controls and blanks to the plate. For details see:

- [Customize Well IDs](#) (below)
- [Customize Sample IDs](#) or assign them additional ID fields on page 217
- [Assign Concentrations or Dilutions to Samples, Standards, Controls](#) on page 218

Customize Well IDs

To save the customized IDs for use in all newly-created protocols/experiments, perform these steps in the Default Protocol at **System > Preferences > Default Protocol**

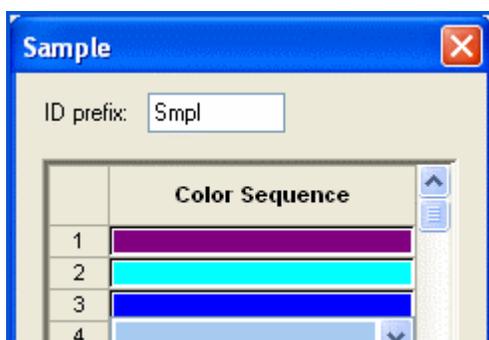
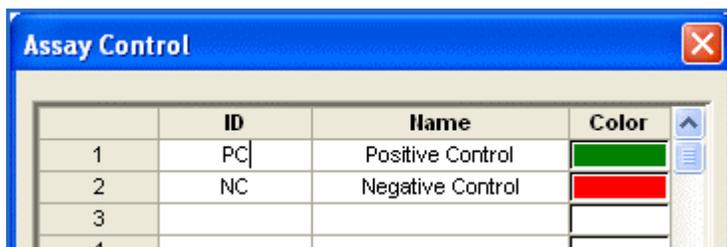
1. Type

- ▼ Use the drop-down list to select the **Type** of specimen, (except for the Empty option, which is used to clear a previously assigned well).

2. ID

Gen5 displays the default label or name for the selected **Type**. Click  (3-dot button) to customize the name and color code. Click in the fields to replace the text and select the color.

Depending on the **Type**, one of three dialogs opens, here are two examples:



- **ID** for Standards, Assay Controls and Blanks can be up to 10 characters, the **ID Prefix** for Samples and Sample Controls can be up to 6 letters, numbers and underscores, without any blank spaces. It cannot end in a number, nor be identical to a formula operand, e.g., X, MIN, MAX, and so on, and cannot be identical to a well coordinate, e.g., A1, H12. Click in the field, and enter text to edit it. The ID can be used in data reduction formulas.
- **Name** can be up to 20 alphanumeric characters and can be used in reports
- The selected **Color** or **Color Sequence** will be applied to the matrix view of the plate. Click in the field to activate the drop-down list of color choices

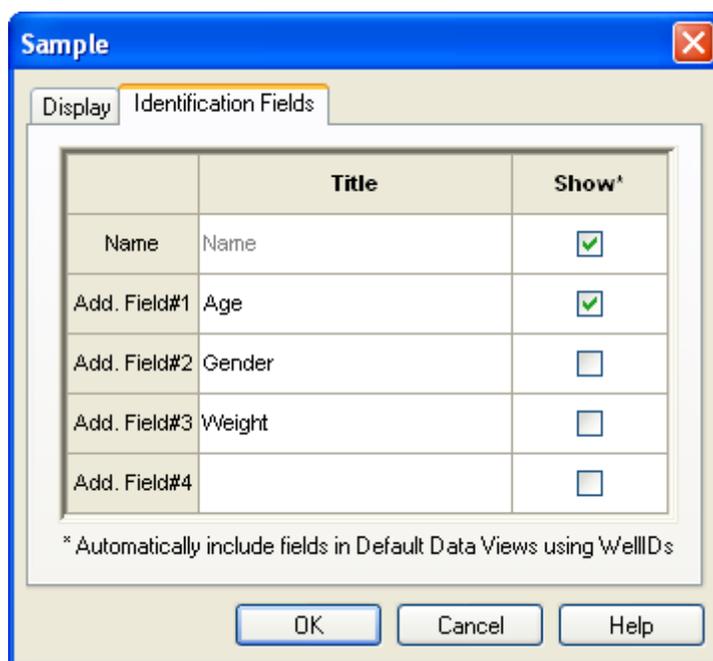
Customize Sample Well IDs

 With **Sample** selected, click the 3-dot button next to the **ID** field to access these controls.

Sample wells are the unknown samples to be processed. To save customized IDs for use in all newly-created protocols/experiments, perform these steps in the Default Protocol at **System > Preferences > Default Protocol**

How to customize Sample wells:

1. On the **Display** tab, click in the field to overwrite the default Samples **ID prefix**: SPL.
The ID can contain letters, numbers, and underscores, up to 6 characters. IDs cannot end in a number and cannot contain spaces. IDs cannot conflict with well coordinates, A1, H12, etc.
2. Click in a **Color Sequence** field to enable the drop-down options, click on the desired color to select it. The first color selected is applied to SPL1, the second color is applied to SPL2, and so on. Gen5 reapplies the colors, starting with the first one, when the entire list has been used.



3. Click the **Identification Fields** tab to create or modify these data categories.
4. For each "Add. Field#" or additional identification field, enter a **Title** or name for this data category.
5. Mark the field to **Show** in Gen5's system-generated data views. This enables the [Matrix data-entry](#) option referenced below.

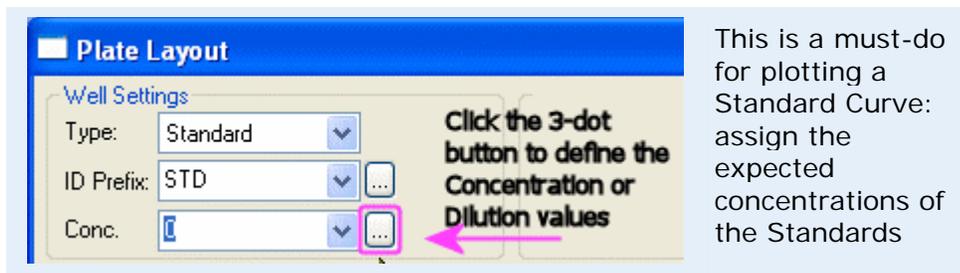
To populate the Identification Fields

The **Title** of the Identification Field becomes a category for data input. For every Sample assigned in the Plate Layout, you can collect the data for each category when you run an experiment. There are two ways to input the data:

- [Fill in the Samples ID Table](#) on page
- [Edit the Matrix to Input Data](#) on page
- Use the Batch IDs creator to "**Apply to Fields**," see page

Assign Concentrations or Dilutions to Standards, Samples, Controls

In the Plate Layout screen, under **Well Settings**, the label changes to either **Conc.** or **Dil.** (for Concentration or Dilution) depending on the **Type** selected and your input.



First, define the concentrations or dilutions, then, assign them to the plate.

Define the Concentrations or Dilutions

After selecting the **Type** and the **ID**, click the 3-dot button next to **Concentrations** or **Dilutions**. When Standards are the selected Type, the cells in this table are labeled accordingly, e.g. STD1 (or STDB1 when [multiple standard curves](#) are created [see the **Data Reduction Options** chapter]). Gen5 applies an indexing notation for all other Types of Well ID, e.g. CTL1:1

1. In ascending or descending order, enter the values in the consecutively-numbered fields. For a shortcut, select one of the two **Auto** entry tools:
 - **Increment** when the dilutions are based on a fixed number to increment your starting entry by. For example, starting at 10, increment by 10 to define concentrations as 20, 30, 40.
 - **Factor** when the dilutions are based on a fixed number to multiply your starting entry by. For example, starting at 1, factor by 10 to define them as 10, 100, 1000 and so on.
 - In the first field, enter the starting number
 - Click in (or use the down arrow key to move to) each successive field to apply the incremented or factored value
2. When the well type is other than Standards, select **Concentrations** or **Dilutions** with the buttons underneath **Conc./Dil. Type**.
3. If applicable, enter an abbreviation for the measurement **Units**.
4. Assign the location of concentrations or dilutions to the plate, in the same manner as assigning Samples. Set the Well Settings and click the well to assign that ID and Conc/Dil value to. Or, use the Replicates and Auto Select options and select a row, column, or click and drag over the area of the plate to assign the IDs.

Helpful Information:

- **Important:** You can assign unique concentration/dilution values to individual Sample, Sample Control Well IDs, and Assay Control groups. For example, SPL1 can be defined as dilutions of 5, 15, and 45, while, SPL2 is defined as dilutions of 10, 25, and 50. Change the ID and click the 3-dot button for Conc/Dil to define a unique concentration/dilution-values table for each individual ID. The values table is not saved for that Well ID until they have been assigned to the plate
- You can define [multiple standard curves](#): described in the **Data Reduction Options** chapter
- In the Plate Layout dialog, you can use the **Dilutions Only/Concentrations Only** button to apply only dilution or concentration values to selected wells without altering the well's identifier
- When assigning concentrations/dilutions, the well selection must be compatible with the **Replicate, Auto Select, and Filling** option settings
- Set up your preferred default IDs and define regularly used concentration/dilution values for Samples, Standards and/or Controls in the [Default Protocol](#)

Custom Plate Layout

Gen5 lets you create individual custom layouts for experiments with multiple plates » not to be confused with [Multi-Plate Protocols](#). Exercise judgement when using this option, keep in mind any Data Reduction formulas that reference Well IDs. You cannot set up Data Reduction steps that reference the custom layout only.

How to create a custom layout:

1. After adding plates, select the  in the menu tree, and right click
2. Select **Custom Plate Layout** from the pop-up menu.
A new **Custom Layout** icon is added to the menu tree for that plate.
3. Double click the plate's **Custom Layout** icon to open the [Plate Layout](#) screen, for that plate only!

Helpful Info:

-  In the menu tree, Gen5 highlights plates with an asterisk to indicate the plate varies from the protocol in some way
- Keep in mind the differences between the Protocol's plate layout in Data Reduction steps, Data Views and Report Builder. For seamless integration of a custom layout, make sure any Well IDs referenced by a Data Reduction step are included in the custom plate

Plate Information

Plate > Information



About the Plate Information

Every plate added to an experiment comes with an **Information** component. The Information is collected when the plate is read. **Runtime Prompts** (described on the next page), set up for the Protocol, define the information requested when the plate is read.

Reviewing the Plate Information

Double click the **Information** element under the Plate in the menu tree to review or modify the information collected when the plate was read.

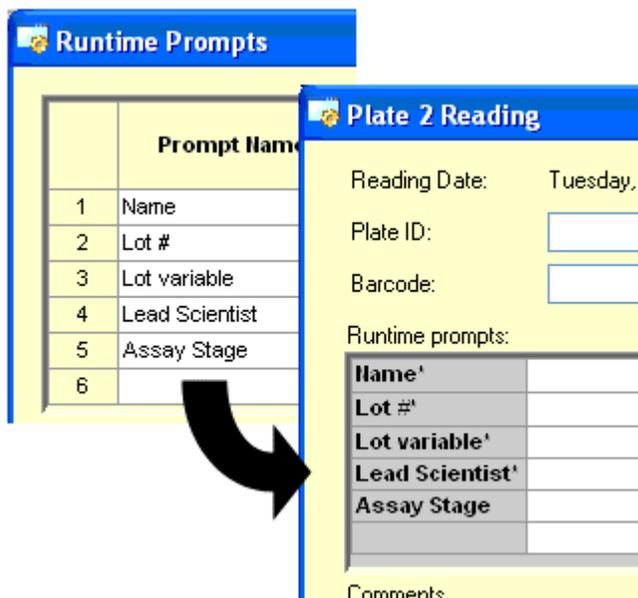
Printing the Plate Information

You can include the **Plate Information** in a report or export file using [Field Groups](#)

Runtime Prompts

Protocol > Runtime Prompts

Use these controls to set up the **Runtime Prompts** presented to users when they **Read** a plate in an Experiment based on this Protocol. The Prompts you define become the labels for input fields in the "**Plate Reading**" screen Gen5 presents when a plate is read. The Plate Reading data is retained in the experiment as **Plate Information**.



The **Runtime Prompts** become input fields in the Plate Reading dialog. **Example:** When the **Prompt Name** is "Name" for prompt 1, users are prompted to enter their name when they read a plate.

- Clear the default text from any unused prompts for the best appearance at run time.

How to:

Find descriptions of these prompt attributes:

- **Prompt Name** on page 223
- **Prompt Type** on page 223
- **Data Reduction Variable Names** on page 224
- **Remember Recent Values** on page 224
- **Skip runtime prompts** on page 225

Reviewing the Plate Information



You can review (and modify) the **Information** entered when the plate was read in the Information component of the Plate in the menu tree

Printing the Plate Information

You can include the **Information** in a report or export file using **Field Groups**

 **Bar-Code Scanning the Plate ID:** If you have a compatible bar-code scanner, you can use it to input the plate's barcode and any other prompts. Replace the keyboard with the scanner and use it to capture the information just before reading the plate.

Creating a Runtime Prompt:

Prompt Name

When defining [Runtime Prompts](#) the Prompt Name becomes the data-input field name in the Plate Reading dialog. Up to 6 prompts can be defined. Prompt Names must be unique or empty, independent of case. LOT and Lot are not acceptable variations, for example.

Field names are limited to 32 characters. Their corresponding data-entry fields support up to 255 characters.

When a prompt is defined as "[required](#)" its Prompt Name is marked with an asterisk in the Plate Reading dialog.

 Delete unused "Prompt #'s" for the best appearance in the Plate Reading dialog. The fields will appear blank instead of displaying the prompt number.

Prompt Type

When defining [Runtime Prompts](#) use the drop-down list to select the Prompt Type:

- **Optional:** gives the user an opportunity to enter data, but the field can be skipped. When all prompts are optional, the Plate Reading dialog can be turned off, blocked from appearing before a plate read.
- **Required:** requires the user to enter data in the field before reading the plate. Required "Prompt Names" are marked with an asterisk in the Plate Reading dialog. Required fields disable the ability to "Skip the Plate Reading dialog."

- **Data Reduction Variable:** requires the user to enter a numeric value for use in data reduction formulas. The entered value replaces the [Variable Name](#), which is used as a placeholder when writing the formulas. Numeric values can be decimal or scientific notation in accord with the computer's Regional Settings. Data Reduction Variables, also called "runtime variables" can be used in these data reduction steps:
 - Transformation
 - Curve Interpolation
 - Cutoff
 - Validation
 - Well Analysis Formula

Data Reduction Variable Names

When defining [Runtime Prompts](#) for Data Reduction Variable Prompt Types, the **Variable Name** is used when writing formulas. The Variable Name is a placeholder for the numeric value Gen5 users are prompted to enter before reading the plate. Although, the value can be modified in the Plate Information dialog at a later time.

At the Plate Reading dialog, Gen5 verifies the user has entered a valid numeric value for the variable. They can be integers, decimals or scientific notation in accord with the computer's "Regional Settings."

Gen5 gives you a headstart when defining a variable name. It repeats the Prompt Name text preceded by an exclamation point (!), because this is the required syntax.

Syntax for Variable Name:

- Must start with "!"
- Other characters must be alphanumeric (a-z; A-Z; 0-9); they are character case dependent
- Up to 32 characters can be used
- Must be unique (to the protocol)

❖ Data Reduction Variable prompts are "required" entry fields.

Remember Recent Values

When defining [Runtime Prompts](#) this option lets you give users a shortcut for data entry. It remembers the last 5 values entered for the prompt and provides them in a drop-down list for easy selection. Gen5 retains the values with the Protocol, so the list is available in any experiment based on that protocol.

❖ Recent Values are managed with the Database Maintenance tools.

Skip Runtime Prompts

You can speed up plate reading by skipping the "Plate Reading" dialog when:

- it is not necessary to collect miscellaneous data about each plate
- none of the Prompts are "Required" entry fields
- none of the Prompts are Data Reduction Variables

Open the Runtime Prompts screen, empty the table is necessary and select

Skip Plate Reading dialog during plate read

Assigning Sample IDs

Plate> Sample IDs

Gen5 provides tools for pairing labeled patient/test samples with their reading and data reduction results. This feature reflects the customization and definition of unknown "Sample" wells in the [Plate Layout](#), e.g. SPL1. For each Sample well assigned in the Plate Layout you can attach an ID or Name, and when you have defined "Additional Fields" you can input related data for each Sample well.

The **Samples IDs** (Names) and other Identification Fields are unique to each test plate. They provide the ability to explicitly relate a test sample to a test subject. Learn more about the Identification Fields that are created in the Plate Layout.

❖ You must be in an **Experiment**, not a Protocol, to assign Sample IDs. And, the Plate Layout must be defined before Sample IDs can be defined and applied to the plate.

You can:

- [Import Sample IDs from a Text File](#) (page 233)
- [Manually Enter Sample IDs](#) (page 226)
- [Batch Sample IDs](#) (page 230)
- [Create a replicate of a previously defined plate](#) (page 233)
- Use a [BarCode Scanner to enter Sample IDs](#) (page 228)
- [Export Sample IDs](#) to a text file (page 234)
- **Print the Sample IDs** after entering them, in list or matrix format. Matrix depicts the plate layout. To include them in a report or export file, add the **Well IDs** table to the Report Content or Export Content in the respective Report or File Export Builder, or using the Power Export toolbar.
- **Clear/Remove Sample IDs** from a plate after they've been assigned: Select **Plate>Sample IDs** and click **Clear All**
- **Delete a column of data:** highlight column in the Samples ID table and press **Delete**

Manually Enter Sample IDs

Gen5 provides three ways to manually enter Sample IDs and data for "Additional Identification Fields":

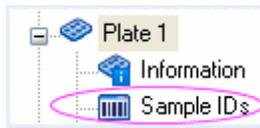
- Fill in the Sample IDs table (described below), or
- Edit the Plate View Matrix (described on page 228), or
- Barcode Scan Sample IDs to enter data (described on page 228)

Helpful Hints:

- Use the [Batch Sample IDs](#) feature for more advanced sample naming described next
- When Sample well identifiers are not consecutively numbered in the Plate Layout, Gen5 does not skip them in the Sample IDs table, but indicates their absence by graying out and putting an asterisk at their label. When automated methods are used to ID samples, Gen5 skips the missing SPL identifiers.

Fill in the Sample IDs Table

❖ This data-entry method is an alternative to [editing the Matrix](#).



1. Locate **Sample IDs** under the **Plate** in the menu tree and double-click to open it.
2. Gen5™ lists the consecutively numbered Sample well identifiers ([SPL](#)) from the **Plate Layout**. Enter their corresponding IDs or names or other data:
 - **Auto Numbering** Select **Auto Numbering** to automatically increment a numeric suffix or a standalone number. For example, enter ABC10 in the first cell of the table and using your down-arrow key or a mouse click, select the next downward cell to assign the next samples ABC11, ABC12, and so on.
 - Use the buttons to **print** the Sample IDs in a List or Matrix format
 - Select the contents of a cell and use Ctrl+C to **Copy** and Ctrl+V to **Paste**
 - Use the **Clear All** button to empty the table: contents of all columns and rows will be deleted. Click **Cancel** to recover data.

 To delete rows, columns or blocks of contiguous cells, select them and click **Delete**. To select a row or column click on its header. Click and drag over a block of contiguous cells to select them.

- **Copy Sample IDs from Another Plate:** Instead of manually entering or importing IDs, when you have multiple plates in an experiment, use the spin buttons or enter the number of the plate you want to copy IDs from and click **Copy**.

Edit the Matrix to Input Sample Data

Gen5 lets you enter Sample Identification Fields data in the Matrix:

Prerequisite:

There are two ways to display the Sample Identification Fields in a Matrix view so they can be input/edited:

- they must be defined to **Show** in the [Identification Fields](#) dialog, this makes them automatically appear in the **Layout** view
- or, they have been included in a custom Data View

How to:

1. With the experiment open and the desired plate selected in a multi-plate experiment, open the Plate View.
2. Select the Matrix tab and from the Data drop-down list select Layout or a custom data view that contains Name or Additional Identification fields.
3.  Click the **Edit** button.
4. Click in a well to reveal placeholders for the fields (if data has not yet been entered). Replace the placeholders or previously entered data with your desired input. Multiple wells can be changed in a session.
5.  To apply the changes, click the **OK** (green check mark) button.
 Click the cancel button to ignore your entries, and restore the original values.

- ❖ Use the right-click options to copy and print the matrix.
- ❖ This data-entry method is an alternative to [filling in the table](#).

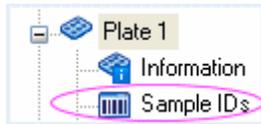
Barcode Scanning Samples IDs

Prerequisite

You must have a wedge-type bar-code scanner that replaces or mimics keyboard input. Follow the manufacturer's instructions for installing and setting up the scanner for carriage return line feed.

- ❖ **Added Bonus:** If you do have a compatible bar-code scanner you can also use it to input a Plate ID and other [Plate Information](#) collected by Gen5 when the plate is read.

How to



1. Open the experiment and locate **Sample IDs** under the **Plate** in the menu tree, and double click to open it.
2. Gen5™ lists the consecutively numbered Sample well identifiers (SPL) from the **Plate Layout**. Use your mouse to select the starting point, e.g. SPL1, and make sure Auto Numbering is de-selected, i.e. unchecked.
3. Operate the scanner according to the manufacturer's instructions to enter the corresponding IDs or names.

Assign Sample IDs for Multiple Plates (Batch)

Plate > Batch Sample IDs

Use the **Batch Sample IDs** screen to assign sample IDs or names to multiple plates simultaneously. **Sample IDs** or names are specific to each test plate, and are intended to relate a test sample processed by Gen5 to a test subject. The batch process offers more advanced auto-numbering methods than the single-plate Sample IDs tool.

Batch Sample IDs

Sample Names

Auto Number
 Use Same IDs for each plate
 Import From File

Base Name:

Initial suffix:

Increment:

Minimum Suffix Width:

Start next plate at the next multiple of

Plates

All
 Selection
 Range to
 Plate 1 Plate 4

Example

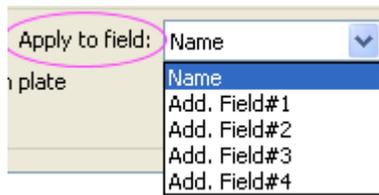
Plate 1	Plate 2	Plate 4
SPL1 Sample1	SPL1 Sample101	SPL1 Sample301
SPL2 Sample2	SPL2 Sample102	SPL2 Sample302
SPL3 Sample3	SPL3 Sample103	SPL3 Sample303
...
SPL48 Sample48	SPL48 Sample148	SPL48 Sample348

OK Cancel Help

❖ The [Plate Layout](#) must be defined before Sample IDs can be applied to the plates.

Auto Number

Select **Auto Number** to manually create a naming/numbering convention for samples for one or more plates:



When you have created additional Sample ID Fields during the Plate Layout, you can use this Batch ID feature to populate these fields. Use the **Apply to Field** drop-down list to select the ID field you want to auto-number.

1. In the **Base Name** text field, enter text that Gen5 will append with a numeric suffix, if desired. Leave the field blank to consecutively number samples without additional text.
2. Enter the **Initial Suffix** — the number to apply to the first sample (of the first plate)
3. Set the **Increment** for sample IDs. Gen5 will leave the defined number of numbers unassigned between each sample. For example:
4. If desired, set the **Minimum Suffix Width** to enforce a uniform appearance to the numbering convention
5. Optionally, set the **Start next plate at the next multiple of** number. Gen5 will increase the next plate's Initial Suffix by the defined "next multiple," as long as that number has not already been used in a plate.

For example: For a **96-well plate**, if samples are numbered consecutively with an increment of 1, and the next multiple is 100, the second plate's samples will be numbered 101, 102, 103, ..., and the third plate's samples will be numbered 201, 202, 203, and so on.

For a **384-well plate**, in the same scenario, the next plate's samples will be numbered 401, 402, and the third plate must begin with 801,

More on Next Multiples: Applying the Next Multiple prefix is determined by the number of samples on the plate and the **Increment** factor. When sample numbering for the plate exceeds 100, and the "next multiple" is set to 100, Gen5 applies the next unused multiple to start numbering the next plate. Thus, for 384-well plates the default next multiple is 1000, which produces the best results for large numbers of samples.

 Watch the effect of your choices in the **Example** space of the screen.

6. In the **Plates** section, define which plates to apply the naming convention to:
 - **All** currently defined plates. This feature will not automatically apply the naming convention to plates added to the experiment after it has been executed. You must execute the feature again when more plates are added to the experiment.

- **Selection** is filled by Gen5 when you select plates in the menu tree before initiating this feature: hold the **Ctrl** key while clicking multiple plates, they will be highlighted. Then, select **Plate>Batch Sample IDs** to see this option selected.
- **Range** to define a contiguous selection of plates

❖ All plates selected for sample naming will be affected, even if [Sample IDs](#) have already been defined for them, i.e. the batch process will overwrite existing IDs.

Use Same IDs for each plate

This option assigns the **same** Sample IDs or names to all selected plates:

1. Click the [Edit Sample IDs](#) button and enter IDs
2. Define the plates to apply sample IDs to in the **Plates** section (see step 6 above for details).

Import From File

Gen5 lets you import a text file of Sample IDs. The file can contain the IDs or names of samples, each one separated by a hard return, and any "Additional" sample identification fields you've defined. When the additional ID fields are included in the text file they must be separated from the Name or Sample ID by the symbol defined in the **Read From File Settings**.

The text file format requires data for each sample to be in a separate row: Name; ID field; ID field, when the "read from file" separator is a semi-colon (;), for example. Use a hard return or "carriage return" to separate each sample's data..

1. **File Name**  Enter the full path and filename or click the 3-dot button to locate the text file containing the Sample IDs for this plate and click **Open**.
2. Define the plates to apply sample IDs to in the **Plates** section (see step 5 above for details).

Beginning with the first text item, Gen5 fills the Samples ID table of the first plate with the corresponding number of IDs. Additional plates are subsequently processed. Extra text is ignored, and insufficient text to fill all the sample cells results in blank name spaces.

Clear Sample IDs from Multiple Plates

You can use the Batch Sample IDs feature to clear or erase the sample names applied to multiple plates:

1. In a multiple-plate experiment, highlight the plates in the menu tree (hold the Ctrl key while selecting the plates with Sample IDs you want to remove)
2. Select **Plate> Batch Sample IDs**, under Sample Names, select **Use Same IDs for each plate**

3. Click the **Edit Sample IDs** button, Gen5 opens the Samples dialog.
4. Click **Clear All** and click **OK**. Gen5 returns to the Batch Samples ID screen.
5. Click **OK**. Click **Yes** at the warning message that all IDs will be cleared.

Create a replicate of a previously defined plate

Gen5 automatically creates a replicate of a plate, when you add a plate to the experiment. The currently-defined **Plate Layout** is applied to the new plate. Gen5 also provides tools for copying Sample IDs from another plate.

Choose your preference:

- Adding (a replicate) plate to an Experiment (see below)
- [Copy Sample IDs from one plate to another](#) on page



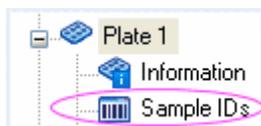
Perform both steps to create an identical copy of the plate!

Import Sample IDs from a Text File

Gen5 lets you import a text file of Sample IDs and "Additional" sample identification fields.

❖ These instructions are for a single plate, for more than one plate see: [Batch IDs](#)

The file can contain the IDs or names of samples, each one separated by a hard return, and any "Additional" sample identification fields you've defined. When the additional ID fields are included in the text file they must be separated from the Name/Sample ID by the symbol defined in the **Read From File Settings**.



1. Locate **Sample IDs** under the **Plate** in the menu tree and double click to open it.
2. Click **Import**. Gen5 opens the standard Windows® open file screen.
3. Locate the text file containing the Sample IDs or names for this plate and click **Open**.

Gen5 assigns the first text item to the first sample well, and fills the Samples ID table with the exact number of IDs. Extra text (i.e. for undefined SPL wells) creates additional cells in the table to hold the extra data. Similarly, when the samples were not consecutively numbered in the Plate Layout, Gen5 does not skip them in the

Sample IDs table, it assigns them an ID. Both extra text and "missing" samples are indicated in the table as grayed out fields.

- ❖ Gen5 offers to delete the extra or "invalid" data/IDs when you modify or review the Samples ID table. Answer **No** to the question: Do you want to keep this data? to delete it.

Import File Format

The import file format requires data for each sample to be on a separate line: Name; ID field; ID field, when the "read from file" separator is a semi-colon (;), for example. Use a hard return or "carriage return" to separate each sample's data. Leave the row/line blank to skip the sample identifier.

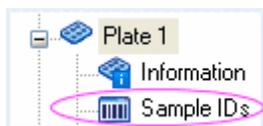
When the "Read from File Setting" is a Tab and one additional ID field is defined the text file for the first 5 samples should look like this, without the title row:

Well Identifier	Sample ID	Additional ID
SPL1	S4532	F
SPL2	S8765	F
SPL3	S2310	M
SPL4	G5876	M
SPL5	T4326	F

The title row is only shown for illustration purposes. It should be omitted from the import file.

Export Sample IDs

Gen5 lets you export a text file of Sample IDs. The file will contain only the IDs or names of samples, each one separated by a hard return.



1. Locate **Sample IDs** under the **Plate** in the menu tree and double click to open it.
2. Click **Export**. Gen5 opens the standard Windows® save file screen.
3. Locate the directory where you want to save the text file containing the Sample IDs for this plate and click **Save**.

Chapter 13

Data Reduction Options

Gen5's robust and flexible Data Reduction options are described in this chapter.

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Well Analysis	280
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Setting up Data Reduction

Protocol > Data Reduction

There are several options available for interpreting the results of your experiment. Gen5™ automatically creates the most commonly applied data reduction steps (based on previously defined Protocol parameters). You can design your own or modify the calculations.

Top 6 Things to Know about Data Reduction

1. Reductions are **StepWise™**, they can be built one upon another, based on their sequence in the Data Reduction dialog: a data set created in a previous step can be used in a later transformation, curve, cutoff, validation or well analysis, if applicable.
2. Gen5 creates four types of data reduction steps automatically:
 - When blanks (BLK) are assigned to the plate, Gen5 automatically creates a **Blank Subtraction** data set. Gen5 subtracts the mean of the blanks from all other wells on the plate.
 - A **Well Analysis** step is created when a multi-read index Read step is defined: Kinetic, Spectrum, Area or Linear Scan. If applicable, blank-subtraction results are used to perform the well analysis.
 - A **Pathlength-Corrected** data set is created when this option is selected in an Absorbance read step.
 - The **Fluorescence Polarization (FP)** transformation is performed automatically when this reading mode is selected in the Read step. If applicable, blank-subtraction results are used to perform the FP reduction. In kinetic experiments, well analysis is based on the FP transformation results.
3. **Important!** Once the Data Reduction dialog is opened and saved, (i.e. OK is selected to close it) Gen5-created reductions are no longer added, deleted, or changed. Keep this in mind when modifying a protocol or experiment.
4. Plate-specific **Data Reduction Variables** can be collected from users when they read the plate so Gen5 can use them in data reduction calculations. First, you define the variable in the **Runtime Prompts**, then, write a formula using the variable name as a placeholder. At runtime, when the measurements and variables are obtained, Gen5 performs the calculation using the input value.
5. Raw data sets used in Data Reduction steps are named according to Gen5's **Data Set Naming Convention**, which is based on the number of **Read** steps defined in the Procedure. When the number of read steps is changed, any previously defined Data Reduction steps are voided, because the data set name is also changed. When you add or remove a Read step, you must update the effected Data Reduction steps.

6. In the Data Reduction dialog, you can:
 - Drag and drop Data Reduction steps to change their sequence order,
 - Select a step and right-click to delete it,
 - Double click an event to open it for modification or deeper review (or right-click and select Edit).

Data Reduction Options

-  Transformation
-  Curve Analysis
-  Well Analysis
-  Cutoffs
-  Validation
-  Fluorescence Polarization

- ❖  **Gen5** shows an invalid data reduction step by blocking out its icon. Changing the **Procedure**, e.g. reading parameters or sequence of events, renaming a Read step or data set, or other changes can invalidate a data reduction step. Generally, it is easiest to delete the invalid step and recreate it, selecting valid options.

How to use a Runtime Variable in a Formula

You can acquire a plate- or assay-specific variable for use in data reduction formulas using the **Runtime Prompts**. When the plate is read, users will be prompted to enter a value that will be used in the calculations.

Follow these steps to write a formula using a **Data Reduction Variable**:

1. Create the Data Reduction Variable in the Runtime Prompts:
 1. Select Protocol>Runtime Prompts
 2. Enter a Prompt Name for the variable: this is the name of the field users will see in the Plate Reading dialog when they read a plate
 3. Set the Prompt Type to Data Reduction Variable
 4. Assign it a **Variable Name**
2. Write the formula: create the applicable Data Reduction Step and include the Variable Name in the formula
3. Save the protocol and create a new experiment based on it.
4.  Read the plate. Enter the value for this runtime variable in the Plate Reading screen that opens just before the plate is read. Gen5 will calculate the formula using the entered value.

- ❖ In **Calibrator-Plate Protocols**, Data Reduction Variables created for and populated by the calibrator plate(s) can be used in data reduction steps defined for the Other Plates. The converse is not true: variables created for the Other Plates cannot be applied to a calibrator plate. Any variable defined for a calibrator plate can be used to create formulas applied to the Other Plates. Variable names must be unique in a protocol, they cannot be reused from plate to plate.
- ❖ In **Multi-Plate Assay Protocols**, Gen5 only recognizes the variable(s) collected for the first plate (Plate 1). Users will be prompted to enter the data reduction variable(s) for every plate, but only the data entered for Plate 1 will be used in the calculation(s).

Define Transformations

Protocol > Data Reductions > Transformations

About Transformations

In Gen5™, the **Transformation** dialog is the workspace to define [plate](#)- and [well](#)-level calculations. If you are familiar with spreadsheets, like Excel, you can write formulas in a similar way.

You can perform calculations using:

- any previously-defined raw data, e.g. measurements obtained from the reader
- already-transformed **data sets**,
- concentrations determined from standard curves,
- well-analysis results
- up to 4 valid data sets

Gen5's **StepWise Data Reduction** lets you perform a Transformation on the results of any previous data reduction step.

The sequence of data reduction steps determines the availability of the data sets: previous steps provide input for subsequent steps. So you can build a series of calculations, defining new calculations to be performed on the results of a previous one.

Well IDs, like blanks (BLK) and standards (STD), etc., must be defined in the plate layout before they can be used in a formula. The matrix in the Transformation dialog reflects the current [Plate Layout](#)

❖ **?????** — This symbol in Transformation results indicates a value could not be determined, or an out-of-range or biased value was used in the calculation. Check the **Protocol Options > Calculation Options** settings.

Basic Steps

The screenshot shows the 'Transformation Dialog' with three highlighted steps:

- Step 1:** 'Data In' is set to 'Read 2:360/40,460/40' and 'Read(s)' is set to 'All'. A button 'Select multiple data sets...' is also visible.
- Step 2:** 'New Data Set Name' is 'Blank Read 2:360/40,460/40'.
- Step 3:** Under the 'Formula' section, the checkbox 'Use single formula for all wells' is checked. The 'Plate Formula' is 'X-BLK'.

At the bottom, a 10x10 data matrix is shown:

	1	2	3	4	5	6	7	8	9
A	BLK X-BLK	BLK X-BLK	SMP1 X-BLK	SMP1 X-BLK	SMP8 X-BLK	SMP8 X-BLK	SMP15 X-BLK	SMP15 X-BLK	SMP22 X-BLK

In the top left corner of the Transformation dialog:

1. Select **Data In**
2. Enter a name for the resulting data set in **New Data Set Name**
3. Enter the formula in the **Plate Formula** field to apply it to the whole plate (all wells) **or** de-select **Use single formula for all wells** and define the **Current Formula** field for individually selected wells: click in the matrix cell to apply the formula to that well of the plate

❖ **Note:** When formulas are applied to individual wells you can **right click** and **Copy** the formula, then **Paste** it into the Current Formula field to see it, reuse it, or modify it. You can also [resize the columns](#) to view formulas.

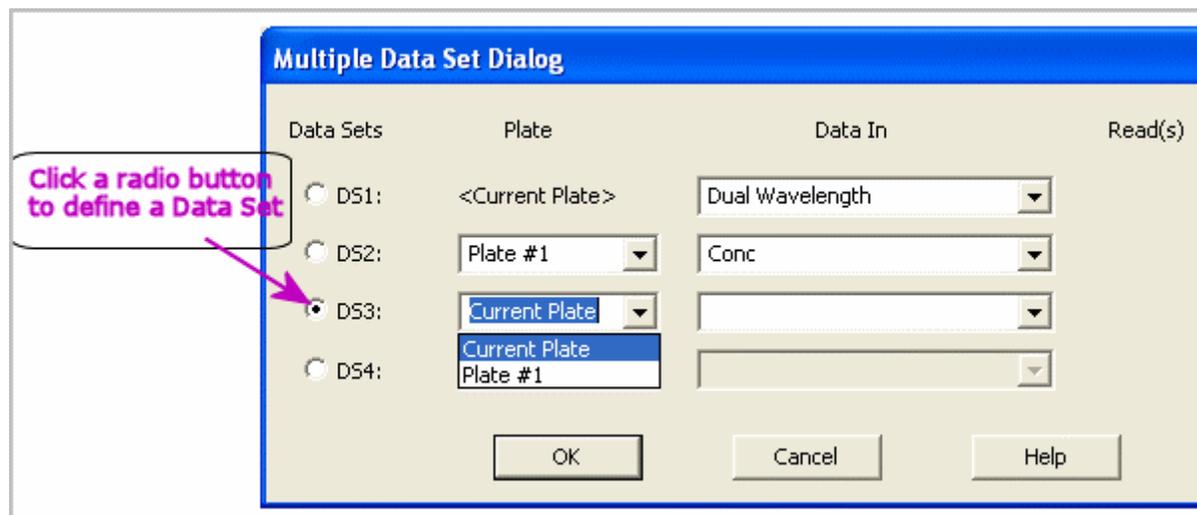
For more details about setting up data transformations:

- [Selecting Multiple Data Sets](#) on page 241
- [Formula Syntax and Examples](#) on page 242
- [Apply calculation to entire plate](#) on page 246
- [Apply calculation to individual wells](#) on page 246

💡 Don't forget to update your [Report](#) to include your transformations.

Multiple Data Sets

In the Transformation dialog, click the **Select multiple data sets...** button:



Use these controls to select the data sets for the Transformation. Click the radio button to enable selection of a data set, and use the drop-down lists to select the content of the data set.

- **Plate:** When multiple plates have been defined for a protocol, you can create a data set using other than the default value of Current Plate
- **Data In:** The drop-down list offers any raw data, or results of previously-defined calculations or curves
- **Read(s):** Kinetic and scanning raw data offers **All** or the results of a specific reading or sampling number to define the data set. Selection is limited in two ways:
 - If you want to use a combination of **All** and individual reads, set **DS1** to **All**, and the subsequent data sets to the individual reads, otherwise, individual read points can be selected for all four data sets
 - When more than one kinetic or other multiple-index data set is available you can only select **All** for more than one data set when their read counts (indexes) exactly match. Otherwise, individual readings from different data sets can be selected.

 When selecting multiple data sets with read indexes, the first data set selected: **DS1**, determines the read index count of the resulting data out: **New Data Set** produced by the transformation.

Transformation Formula Syntax and Examples

Here are the Symbols and Functions that can be used in **Transformations**, in formulas applied to the whole plate or individual wells.

Symbol	Description	Example
x or X	Represents the current well value	
Well Coordinates	Represent the value of a particular well	A2 or H12
Multiple Data Sets (DS#) <data set>.<well>	When referencing multiple data sets use the DS#. To identify a specific well within a data set, use a period to separate the data set and the well coordinate	DS1.H6 = well H6 of data set 1 DS1.X or DS1 = current well of DS1
<well>.<plate>	When referencing a well coordinate in a multi-plate experiment, identify the specific well and plate using a period	B3.3 = well B3 on Plate 3 DS1.H6.2 = well H6 of data set 1 on Plate 2
Well Identifiers wellID_ALL	The value of a specific well. The ID assigned to a specific well, including a Conc/Dil index value, if applicable: <ID><index>:<Conc/Dil Index> The last number is the Conc/Dil <u>index</u> , not the concentration/dilution value. The well index can be replaced by _ALL for Samples and Sample Controls, which returns the mean of all indexes of the well type. Does not apply to STD, BLK or Assay Controls	SPL3 SPL_ALL SPLC_ALL:3 = the mean of SPLC at the 3rd conc value STD3.3 = STD3 on Plate 3
<wellID>:<conc index>	Use : as a separator to identify individual well IDs of a specific concentration/dilution level.	CTL2:3 = the average value for all CTL2 at the third concentration or dilution level
Data reduction variable	Represents a value collected with the Runtime Prompts	!KitFactor
Function Operators	Add +, subtract -, divide /, multiply *, combine ()	CTL3+H5, STD3-25, DS1/DS2, SPL_ALL*2, (STD1/STD6)*100

Figures/Scientific notation	Any numeral, including those expressed with scientific notation	2.45E-08
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Function	Description	Example
Mean(<ID>) Mean(x;y;z;...)	Represents the mean of the specified well identifier or variables; the mean of any set of variables can be expressed	Mean(SPL1;SPL2;SPL3) Note: Gen5 automatically uses the mean of all like-named wells on the plate
SD(<ID>) SD(x;y;z;...)	Represents the standard deviation of the specified well ID or variables	SD(SPL10) SD(23;75;45)
CV(<ID>) CV(x;y;z;...)	Represents the coefficient of variation for the specified well ID or variables, expressed as a percent	CV(CTL2) CV(1;2;3)
DIL(<ID>) or Dil(X); CONC(X) or Conc(<ID>)	Returns the defined dilution or concentration of the specified well ID or current well	Dil(CTL3) CONC(STD2) Conc(X)
Round(x;y)	Rounds x to the y number of significant digits. x can be any valid symbol or expression.	Round(X;2) (X represents the value of the current well)
Truncate(x;y)	Truncates x to the y number of significant digits. x can be any valid symbol or expression.	Truncate(CTL3;3)
Log(x)	Represents the Log10 function	Log(SPL_ALL)
POW(x;y)	Represents the value of x raised to the power of y	POW(STD1;2)
POW(10;x)	Calculates the Anti-Log of the current well	POW(10;x)
SQRT(x)	Represents the square root of x	SQRT(A1*B1)
MIN(x;y;z;...)	Returns the minimum of the defined variables.	Min(CV(SPL1);CV(SPL2))
MAX(x;y;z;...)	Returns the maximum of the defined variables.	MAX(A1;B1;C1)

Functions allow a combination of expressions	(x;y) Any expression that represents a single value, including well identifiers, locations, numerals, a function that results in a single value, can be included in the formula, if it's a valid expression. Functions described with the ellipsis (x;y;z;...) allow up to 10 expressions.
----------------------------------------------	----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

❖ Well IDs are case sensitive, i.e. they must be entered exactly as they are defined in the plate layout. Other symbols and the functions are case insensitive.

Formula Examples

Formula	Description
$X + (A5 + A6) / 2$	The result of this formula is the sum of the current well value and the mean of the A5 and A6 wells.
$DS1 / DS2$	This is the ratio calculation of data set 1 and data set 2.
$\text{Log}(X / PC)$	The result of this formula will log the ratio of the current well over the mean of PC (user-defined identifier).
$\text{MAX}(A1; A2; A3) - \text{MIN}(B1; B2; B3)$	This formula calculates the difference between the maximum value of A1, A2, A3 and minimum value of B1, B2, B3.
$\text{CTRL1} - 3 * \text{SD}(\text{CTRL1})$	The result of this formula is the mean of the wells containing the identifier CTRL1 minus three Standard Deviations of these wells. It is the equivalent of $\text{MEAN}(\text{CTRL1}) - 3 * \text{SD}(\text{CTRL1})$
$X * \text{DiI}(X)$	The result of this formula is the current well value times its dilution factor
$((\text{SPL4} - \text{BLK}) / (\text{SPLC4} - \text{BLK})) * 100$	This formula can be used for toxicology assays, in this example to calculate the toxicity percentage of SPL4.

More on Min/Max

The MIN/MAX calculations are limited to 10 arguments, but by combining them you can apply up to 100 arguments:

- 1 level: $\text{MIN}(A1; B2; C3; D4; E5; F6; H7; A8; B9; C10)$ works for 2 to 10 arguments
- 2 levels:
 $\text{MIN}(\text{MIN}(A1; B2; C3; D4; E5; F6; H7; A8; B9; C10); \text{MIN}(F1; G2; H3; A4; B5; C6; D7; E8; F9; H10); \dots)$ works for up to 100 arguments if you use 10 MIN statements inside a higher level one.

❖ **Note:** you cannot use Min/Max for one Well identifier, like Min(STD1) or Max(NC). You must use the well locations to determine the min and max of a well ID's replicates.
For example: If you use "MIN(NC;POS)", this translates as "MIN(MEAN(NC);MEAN(POS))" and does not return the value that corresponds to the minimum value of all wells where you either put NC or POS. It returns either MEAN(NC) or MEAN(POS), whichever is lower.

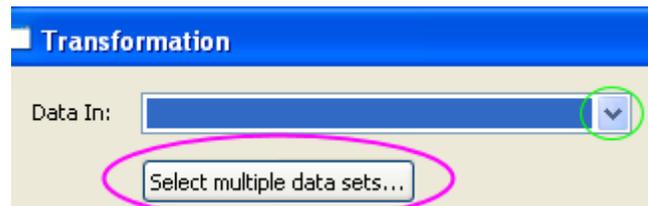
Round or Truncate to control results

While Gen5 provides a way to control the number of significant digits or decimal places to display in reports and on-screen, when performing data reduction operations, Gen5 uses all the digits (up to 15) regardless of the numeric format applied for display. Use the **Round(x;y)** and **Truncate(x;y)** functions to control the number of digits used in and/or generated by a calculation.

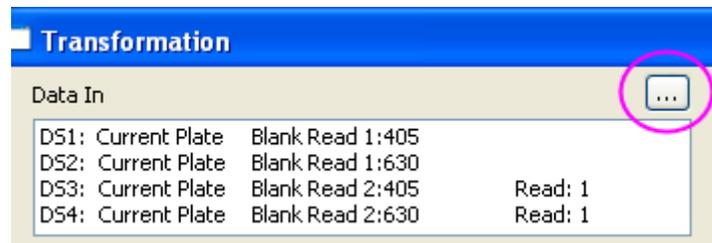
Adding and Selecting Data Sets

Protocol > Data Reduction > Transformation

Gen5™ provides enormous flexibility in designing data reductions by letting you build up a series of data sets. The variables or data sets for your calculations can be raw data or the results of a previously-defined Data Reduction step.



When one or no data sets selected



Multiple data sets already selected

The Transformation dialog changes when multiple **Data In** data sets have been selected, as demonstrated above. Initially, the dialog offers the **Select multiple data sets** button. Use the drop-down list to select one data set. Click the button to select multiple data sets.

When multiple data sets have been selected, the selected data sets (DS1, DS2, up to 4) are displayed with a **three-dot** button.



Both buttons lead to the [Multiple Data Sets](#) dialog, where you can select from raw data or results data from previously defined transformations, well analysis formulas and curves.

Apply calculation to entire plate

Select **Protocol > Data Reduction > Transformation** and retain the default settings to apply a formula to all the wells of the plate:

Formula

Use single formula for all wells

Plate Formula:

In the Formula section of the **Transformation** dialog, affirm with a check mark "Use single formula for all wells."

In the Plate Formula field, enter the calculation [formula](#) to apply to all wells.

Apply calculation to individual wells

Select **Protocol > Data Reduction > Transformation** and de-select "Use single formula for all wells." This changes the input-field label from Plate Formula to **Current Formula**.

1. First, de-select **Use single formula for all wells** by clicking the checkbox to remove the check mark.
The formula field label changes to **Current Formula** and additional auto-entry options are enabled:
 - Current Formula, selected by default, refers to the formula entered in the field
 - Difference Between Columns
 - Difference Between Rows
2. In the **Current Formula** field, enter a formula to apply and then select (click in) one or more wells to apply that formula to them.



Helpful Hints:

- **Right click** a well or field for tools: **Copy, Paste, Cut, Undo**
- Use the **Undo** button (which retains 10 past actions) to reverse an action
- **Resize** the window and/or the grid's columns to see the formula written to a well; (the formula is truncated when it is too long to display):
 - Hover the mouse between two columns to engage  (the resize tool), click and drag to the desired column width
 - Resize the window using standard Windows® tools: click and drag the two-headed arrow icon at the window's corners or edges

- **Reference specific wells** in a formula: often, after creating a data set based on individual-well formulas, it is necessary to reference the specific well or wells in a subsequent transformation using that data set.

Correcting a formula

Use the right-click pop-up menu if you need to make small corrections to a previously-applied formula:

1. Right click as you're highlighting or selecting the well to be fixed
2. From the pop-up menu select **Copy**
3. **Paste** the formula in the **Current Formula** field, with a right click or Ctrl+V
4. Make the needed changes to the formula
5. Apply the revised formula to the desired cell with a regular left click.

Individual-Well Formula Example 1

Here's an example of the need to reference a specific well in a transformation formula.

An assay kit requires subtracting the average of the "non-specific binding" wells (**NSB**) from the "maximum binding" wells (**MB**) to determine the corrected maximum binding, which is used in a subsequent transformation.

The wells to be referenced are assigned to the first column of the plate layout:

	1
A	BLK
B	BLK
C	NSB
D	NSB
E	MB
F	MB
G	MB
H	SPL1

Transformation 1: $MB - NSB = DS1$ (data set 1). This formula can be applied to the whole plate, even though only the specifically referenced wells will be affected by it. Viewing the output data set shows the results, the corrected maximum binding, are assigned only to the applicable wells: C1, D1, E1, F1, G1.

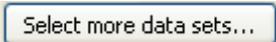
Transformation 2: $X - NSB / DS1.C1 * 100$ can be applied to the whole plate or individually to the sample wells. Notice the reference to well C1 of the data set (DS1), this could have been any of the relevant wells, i.e. referencing F1 would also work.

How to perform Dual-Wavelength Subtraction

A common way to improve the accuracy of your results is to read the plate at two wavelengths and perform a dual-wavelength subtraction data reduction. Here are step-by-step instructions for [endpoint](#) and [kinetic](#) (multi-index) reads:

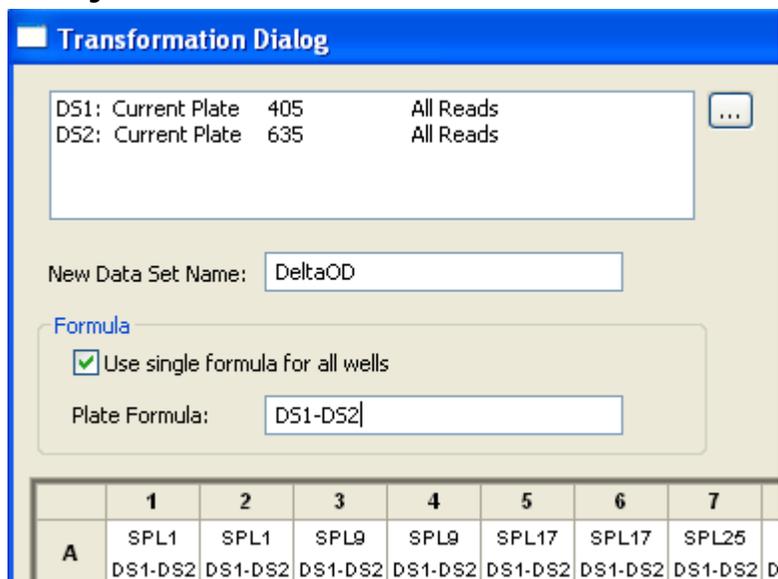
Endpoint Analysis

First define the **Read** step with two wavelengths:

1. Select **Protocol>Procedure** and add steps as needed, e.g. Set Temperature
2. Click **Read** and select 2 wavelengths
3. Define the **Plate Layout** of samples, blanks, standards, etc.
4.  Select **Protocol>Data Reduction** and click **Transformation**
5.  Gen5 opens the **Multiple Data Set** dialog
6.  Select the button for **DS2**
7.  Use the drop-down list to select **Data In** for **DS1** and **DS2**, the first read measurement for DS1 and the second read data set for DS2. Click OK when you're done.
8. In the Transformation dialog, enter a name for the **New Data Set Name** in the text field, e.g. Delta OD
9. Define the dual wavelength subtraction formula in the **Plate Formula** text field, e.g. DS1-DS2

Now you can use the results of this calculation, Delta OD, in subsequent data reduction steps, if desired.

Kinetic Analysis



The screenshot shows the Transformation Dialog box. At the top, it lists two data sets: DS1: Current Plate 405 All Reads and DS2: Current Plate 635 All Reads. Below this, the 'New Data Set Name' field contains 'DeltaOD'. The 'Formula' section has a checked box for 'Use single formula for all wells' and the 'Plate Formula' field contains 'DS1-DS2'. At the bottom, a table shows the plate layout for row A, with columns 1-7 containing sample IDs (SPL1, SPL1, SPL9, SPL9, SPL17, SPL17, SPL25) and the calculated formula DS1-DS2 for each well.

	1	2	3	4	5	6	7
A	SPL1	SPL1	SPL9	SPL9	SPL17	SPL17	SPL25
	DS1-DS2						

First define the **Read** step with a kinetic loop and two wavelengths:

1. Select **Protocol>Procedure** and add a **Kinetic** loop and other steps as needed, e.g. Set Temperature
2. Click **Read** and select 2 wavelengths
3. Define the **Plate Layout** of samples, blanks, standards, etc.
4.  Select **Protocol>Data Reduction**. Gen5 opens the StepWise™ Data Reductions dialog
5. Highlight the first Well Analysis step and click **Transformation**. StepWise Data Reductions let us use the results from all previous calculations in subsequent steps. By making the dual-wavelength subtraction the first step we can use the results in all other steps. (Later, we can modify the Gen5-generated Well Analysis steps to use the dual-wavelength results.)
6.  Gen5 opens the **Multiple Data Set** dialog
7.  Select the button for **DS2**
8.  Use the drop-down list to select **Data In** for **DS1** and **DS2**, the first read measurement for DS1 and the second read data set for DS2.
9.  Set the **Read(s)** for both DS1 and DS2 to **All**. Click OK when you're done.
10. In the Transformation dialog, enter a name for the **New Data Set Name** in the text field, e.g. Delta OD
11. Define the dual wavelength subtraction formula in the **Plate Formula** text field, e.g. DS1-DS2

Now you can use the results of this calculation, Delta OD, in the Well Analysis data reduction steps, if desired. In the StepWise Data Reductions dialog, to modify the Gen5 generated steps, double click a step to open it in edit mode, and change the **Data In** to Delta OD. Or, create a new well analysis step.

Subtracting Blank Wells

When there is one or more **Blank** defined in the Plate Layout, Gen5™ automatically creates a Transformation or "blanked" data set.

❖ Gen5 uses the "blank" (blank-subtracted) data sets in any subsequent system-generated data reduction steps, like Pathlength Correction and **Well Analysis** calculations in kinetic experiments.

For single- and multi-wavelength reads:

For each raw data set, Gen5:

1. Calculates the mean of the raw measurement values in wells identified as blank
2. Subtracts the mean from the raw measurement value in each well on the plate to generate a "Blank" data set containing each well's measurement result after blank well subtraction

3. Displays the **Blank** well Mean in the **Statistics** data view.

For Specific Read Types:

Within any protocol, if one or more wells are defined as 'Blank' in the Plate Layout, Gen5 automatically generates Blank-Wells Subtraction data sets for each wavelength:

- **Endpoint** read: For each wavelength defined in the protocol, the average of the blank well(s) is subtracted from every well on the plate
- **Kinetic** analysis: For each wavelength defined in the protocol, within each kinetic read, the average of the blank wells is subtracted from every well on the plate
- **Spectrum scan**: A blank average is calculated for each wavelength in the spectrum reading range. The blank average for each wavelength is then subtracted from the absorbance read at the corresponding wavelength in each well
- **Linear and Area scan**: For each wavelength defined in the protocol, within each read index, the average of the blank wells is subtracted from every well on the plate

Plotting a Curve

Data Reduction > Curve Analysis

Select **Curve Analysis** in the **Data Reduction** dialog to create a standard or titer curve for your experiment. Other than kinetic curves, there are two scenarios for using a curve to determine the concentration of samples:

Gen5™ provides two general ways to use its curve plotting feature:

- **Standards, Controls, and/or Samples**, for which concentrations or dilutions have been defined, are read on one or more microplates, along with the test/unknown samples.
- A **Multi-Plate Calibration Protocol** defines one plate as the calibrator (containing the Standards or Controls), and plots a curve against the calibrator plate to determine the concentration of test/unknown samples on one or more other plates. Refer to the **Multi-Plate Protocols** chapter for details.



Gen5 lets you generate multiple standard curves (up to 6) from one plate

Prerequisites for generating a Curve:

1. Define **Standards (STD n)**, **Controls (Assay or Sample)**, and/or **Sample Dilutions (SPL)** in the Plate Layout with corresponding concentrations or dilutions ([Learn about Multiple Standards](#) on page 272)
2. Define the minimum number of standards/dilutions for the desired Curve Fit (**Minimum STDs** on page 259)
3. Create at least one read step in the Procedure.

To View a Curve:

After you've defined the Curve Analysis, and acquired the data, e.g. read the plate, open the Plate View, and select the **Graph** tab.

- **Curve Fitting Results:** The default table displays the parameters used to plot the curve including the **Curve Formula**, its elements and the R^2 coefficient (for all curve fits except point-to-point).
- **Curve Fitting Details:** Select the Details table to view best-fit results for each parameter, including standard error (SE) and 95% confidence intervals.
- **Curve Interpolations:** When one has been defined for the curve, Gen5 builds a table listing the interpolation formula and the X and Y values for that point on the curve.

To Output a Curve:

Gen5 provides several ways to print a curve:

-  Click **Quick Export** to instantly send the current curve to Excel
- Add it to the Content in [Report Builder](#)
- Right-click to copy or save it as an image for use in a word-processor application
- [Export it to Excel®](#) using Power Export

How to Create a Standard Curve

Gen5 lets you create one or more standard or calibration curves for determining the concentration of samples:

1. Select **File > New Protocol**
2. Select **Procedure** and define the **Read** step (and any other required steps)
3. Select **Plate Layout**:
 - Define the concentrations of the **Standards** (see below)
 - Assign the location of the standards, samples, and controls and blanks (if any) on the plate
4. Select **Data Reduction > Curve Analysis**
 - Gen5 may have generated a "corrected" data set: if you assigned blanks to the plate or selected Pathlength Correction in the Read step, you'll want to select these data sets for **Data In** for the **Y-Axis Data** when plotting the curve
 - For Kinetic reads, Gen5 creates a Well Analysis step. The default kinetic well analysis is "Mean V," which can be easily changed to any of the wells analysis options. The output of the well analysis step is typically used as the **Data In** for the Y-Axis in quantitative assays.
5.  On the [Data In](#) tab, use the drop-down to select the **Y-Axis Data**
6. On the [Curve Fit](#) tab, choose a curve fit method
7. Other options and requirements when defining multiple curves:
 - **Curve Name**: replace the default "Curve" with a more meaningful or unique name
 - On the [Data Out](#) tab, replace the default "Conc" for the **Data Set Name** with a more meaningful or unique name
 - On the Data Out tab, define interpolations to plot on the curve
8. Define the reporting or export requirements and **Save** the protocol. Now, you're ready to run an experiment: **File > New Experiment** to read the plate and generate the curve.

Define Standards



In the **Plate Layout** dialog:

1. Set the **Type** to **Standard**
2. Gen5 sets the **ID** to STD:
 - You can change the abbreviation using the 3-dot button
 - Use the drop-down list to [define multiple standards](#).
3. Click the 3-dot button at the **Conc.** field to enter the expected concentrations for the Standards:
 - In ascending or descending order, enter the values in the consecutively-numbered fields: STD1, STD2... For a shortcut, select one of the two **Auto** entry tools

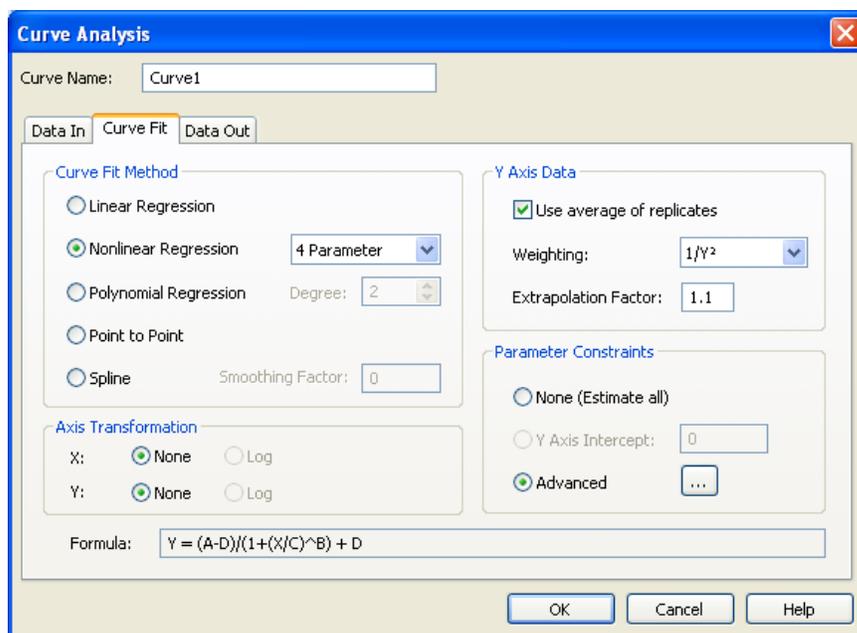


Be sure to assign the [Minimum Number of Standards](#): see page 259

4. Assign the location of the Standards on the plate, in the same manner as assigning unknown Samples.

Curve Fit

Data Reduction > Curve Analysis > Curve Fit tab



Curve Name: assign each curve a unique alpha-numeric name that does not include spaces or symbols used as mathematical operators (+, *, -, /). Characters must be alphabetic, numeric, or the underscore.

Select the **Curve Fit Method** that will best model the data for your experiment. The parameters selected for Curve Fit can be modified at any time without invalidating the data. You may want to experiment with the options to assess the best method for your experiment.

❖ If the options on the Curve Fit tab are disabled (grayed out), **Data In** is set to "Use Curve from Calibrator Plate," which is only applicable to [Calibration Plate Protocols](#).

Gen5 offers several curve fit methods and lots of flexibility in defining or influencing the best-fit calculation. All the options are intended to fit data to a model that defines Y as a function of X, i.e. X values must be known. Gen5's StepWise™ Data Reduction feature lets you transform the data prior to fitting the curve, if necessary. And, you can apply constraints to the parameters or weighting to the sum-of-squares after viewing the curve, as well as before it is plotted.

Linear Regression

Linear Regression is a simple, best-fit, straight line. See page 255.

Polynomial Regression is an extension of the Linear Regression equation. See page 258

Non-Linear Regression

4 Parameter is characterized by a symmetrical sigmoidal plot that eventually becomes asymptotic to the upper and lower standard values. See page 256.

5 Parameter is similar to 4P but it is better able to model data that is asymmetrical at the upper and lower asymptotes. See page 257.

Logit-Log is a restricted form of 4P that has been offered in BioTek readers' onboard data reduction (PDR) software and KCjunior™. See page 258.

Other

Point-to-Point follows each standard point with no averaging of the values to smooth the curve. Minimally two standards are required.

Spline with Smoothing is a curve defined piecewise by polynomials, joining a set of data points by a series of straight lines, which is then smoothed by the **Smoothing Factor**.

The curve will appear smoother as the Smoothing Factor is increased. The zero factor will force the curve fit through all data points.

This curve is most useful when you have a very large data set. Minimally 4 standards are required to plot a Spline curve.

Linear Regression

Linear regression: $y = ax + b$ (or $Y = \text{intercept} + \text{slope} \times X$).

y represents unknown response

x represents theoretical concentration (in this context should we be using the word expected?)

a represents the slope of the linear regression

b represents the intercept to the y axis

- The linear regression fit uses the least squares technique. The better the quality of the fit the more the absolute value of R tends to 1. This type of curve fitting technique can be used when you think the data will fall in a predictable linear pattern. It may be necessary to transform the **x** and/or **y** components using **Logarithmic axes**.
- You can enable or disable **Use average of replicates** in the curve calculation. By default, the mean of replicates is used. This is recommended when the replicates are not independent. Contrarily, when the source of experimental errors is the same for all replicates, you should consider plotting each point separately.
- You can apply **Parameter Constraints**, but generally, linear regression determines the best-fit curve without constraints.

Nonlinear Regressions are Iterative

Nonlinear regression is performed in a series of steps, each adjusting the parameters to improve the goodness-of-fit.

Here are the steps that every nonlinear regression program follows:

1. Start with an initial estimated value for each variable in the equation.
2. Generate the curve defined by the initial values. Calculate the sum-of-squares (the sum of the squares of the vertical distances of the points from the curve).
3. Adjust the variables to make the curve come closer to the data points. There are several algorithms for adjusting the variables. Gen5 uses the Newton-Raphson method.
4. Adjust the variables again so that the curve comes even closer to the points.
5. Keep adjusting the variables until the adjustments make virtually no difference in the sum-of-squares.
6. Report the best-fit results. The precise values you obtain will depend in part on the initial values chosen in step 1 and the stopping criteria of step 5. This means that repeat analyses of the same data will not always give exactly the same results.

4 Parameter Curve

The four parameters are left asymptote, right asymptote, slope, and the value at the inflection point. Gen5 performs an iterative series of calculations (using the Newton-Raphson algorithm) to determine the best curve fit - the least squares method to minimize the residual error. The iterations stop when no further improvement to ERR is detected. You can refine the calculation using Parameter Constraints and/or [Weighting](#).

4 Parameter logistic fit:

$$y = \frac{a-d}{(1+(x/c)^b)} + d$$

a = (theoretical) response at concentration = 0

b = measure of slope of curve at its inflection point

c = value of **x** at inflection point

d = (theoretical) response at infinite concentration

x = concentration

y = response (OD)

The minimum number of standards is 4.

❖ IC_{50}/EC_{50} : typically, the **c** parameter is equal to the IC_{50} or EC_{50} value

It is recommended that:

- at least one standard is not far from each asymptote
- at least 2 standards fall within the linear area of the curve, one either side of the inflection point

 You can plot the IC_{50}/EC_{50} value on the 4P curve by updating Gen5's Curve Analysis step before reporting the results.

5 Parameter Curve

As you would expect, the five parameter curve is nearly identical to the 4 Parameter except for an additional parameter **e** (which is equal to 1 in a 4P curve and makes the curve symmetrical). With **e**, the 5P curve fit is better able to model asymmetric experiment results. You can refine the calculation using Parameter Constraints and/or [Weighting](#). Gen5 ends the iterative calculations when no further improvement to ERR is detected.

❖ This five-parameter logistic is also called the Richards equation.

$$y = \frac{a-d}{(1+(x/c)^b)^e} + d$$

a = minimal curve asymptote; (theoretical) response at concentration = 0

b = measure of slope of curve at its inflection point

c = value of **x** at inflection point

d = maximal curve asymptote; (theoretical) response at infinite concentration

e = quantifies the asymmetry

x = concentration

y = response (OD)

The minimum number of standards is 5.

It is recommended that:

- at least one standard is not far from each asymptote
- at least 3 standards fall within the linear area of the curve, one at the inflection point and one on either side of it.

 You can calculate and plot the IC_{50}/EC_{50} value on the 5P curve by manually performing the calculation and then updating Gen5's Curve Analysis step before reporting the results.

Polynomial Regression Curve Fit

The calculation of the Polynomial fit parameters is based on a least squares method that results in a series of equations. Then, a Gaussian Elimination algorithm is applied to the augmented matrix of the series to calculate the parameters.

$$y = a_n X^n + a_{n-1} X^{n-1} + \dots a_1 X + a_0$$

n represents the **Degree** of the polynomial regression.

- The **Degree** must be less than or equal to the number of standards minus 1. If this condition is not met, Gen5 automatically reduces the degree of the polynomial to the number of defined standards having different X values minus one.
- The unknown concentrations are calculated by using an approximate calculation method, linear interpolation between 1000 points evenly spaced on the X axis.
- When a **Y Axis Intercept** value is defined it determines the coefficient a of the polynomial equation:
 $y = g*x6 + f*x5 + e*x4 + d*x3 + c*x2 + b*x + a$
 This is equivalent to defining an advanced parameter constraint with a fixed.

Logit-Log Curve

Gen5's Logit-Log curve is identical to the [4 Parameter Curve](#) except it does not perform an iterative series of calculations to minimize residual error. Instead, the asymptotes of the curve (parameters a and d) are determined from experimental data (y values). And, the inner portion of the curve (b and c) is solved using a logit-log linear regression. This prohibits the application of Parameter Constraints and Weighting.

❖ Gen5's logit-log algorithm is derived from BioTek readers' onboard data reduction (PDR) software and KCJunior™.

Curve Fit: Minimum Number of Standards

Here are the minimum number of standards that must be defined for each **Curve Fit Method**:

Curve Fit Method	Minimum STDs
Linear Regression	2
4-Parameter	4
5-Parameter	5
Logit-Log	4
Point-to-Point	2
Spline with Smoothing	4
Polynomial Regression	Degree + 1
Degree: 2	3
Degree: 3	4
Degree: 4	5
Degree: 5	6
Degree: 6	7

More Curve Fit Controls:

Axis Transformation:

In the bottom left corner of the Curve Fit tab, you can alter the default Transformation for the X and Y axes used to calculate the curve.

- **None** - the default value, retains the **Formula** displayed at the bottom of the dialog to calculate the curve (no transformation is applied to the selected data, X or Y.)
- **Log** (logarithmic) - alters the formula used to calculate the curve by applying $\log_{10}(10x)$ to the selected data, X or Y. Any change is reflected in the **Formula** displayed at the bottom of the screen. Log transformations fail when the data includes a negative or null value. And, they are not applicable to the Nonlinear Regression curves.

Y Axis Data:

Use average of replicates

This check box offers a Yes or No option to calculate and apply the average of replicates when plotting the curve or to calculate each data point individually. The

default setting is yes, to average the replicates, but some types of assays consider results to be more accurate when each sample is plotted separately. This option is disabled for Point-to-Point and Spline curves.

 **Weighting:** Use the drop-down list to apply a weighting factor to the curve fit formula. (See below for more info.)

1.1 **Extrapolation Factor:** The extrapolation factor range must be between 1 and 3. (See below for more info.)

Parameter Constraints:

- None (Estimate All)
- **Y Axis Intercept** is limited to Linear and Polynomial Regression fit methods. This option forces the curve to intercept the Y axis at the value you input. See page **Error! Bookmark not defined.**
- **Advanced** : Click the 3-dot button to define constraints for the curve. See page 262.

Weighting in a Curve Fit

Gen5 offers three options for weighting the curve fit to normalize or minimize-the-effect of an uneven distribution of data points from the curve. Select the most appropriate option for your experimental data:

[1/Y - Poisson Weighting](#)

This weighting option is available to refine data that follows a Poisson distribution. Where the standard deviation among the replicates is almost equal to the square root of their mean.

$$\sum \frac{1}{Y_{data}} (Y_{data} - Y_{curve})^2$$

[1/Y² - Relative Weighting](#)

When you expect (or discover) the average distance of the points from the curve to increase as Y increases, you can use this weighting option to minimize the sum-of-the-square of the relative distances. Relative weighting can ensure that all points have an equal influence on goodness-of-fit.

$$\sum \frac{1}{Y_{data}^2} (Y_{data} - Y_{curve})^2$$

[1/Std Dev Y² - Reciprocal-Variance Weighting](#)

When you have a large range of data values, variable error in the data or a relatively large error in the data you have good reason to consider applying a weight to each data point. This method of "weighting by observed variability" assumes that the mean of replicates with a large standard deviation (std dev) is less accurate than the mean of replicates with a small std dev. This is not always true.

This method is only reliable when you have a large number of replicates.

$$\sum \frac{1}{SD^2} (Y_{data} - Y_{curve})^2$$

- ❖ The setting: **Protocol > Protocol Options > Calculation Options > Standard Deviation Weighting** N-1 or N is used to determine SD prior to its use in this weighting scheme.
- ❖ At times Gen5 may not be able to calculate one or more weights. Calculation Warning messages will alert you to the situation and describe the remedy Gen5 implemented, e.g. the highest valid weight was used

Extrapolation Factor in Curve Fitting

For all curves except Spline and Point-to-Point, you must define an Extrapolation Factor in the Curve Fit tab or use the default setting: 1.1. The Extrapolation Factor must be between 1-3.

Using a factor of 1 eliminates the extrapolation, using the upper and lower Standards as the limits for concentration/dilution interpretation. An Extrapolation Factor >1 lets you extend the upper and lower limits of the calculation.

- **Example:** Assuming a linear X axis, we wish to extend the upper and lower limit by 20%. The lower limit denoted by the first standard is 50 units. The upper limit denoted by the last standard is 150 units. The dynamic range therefore is 150-50 = 100 units. Imposing the 20% extension means that the new range will be: 100 x 1.2 = 120 units. This represents an increase in the dynamic range of 10 units at the lower limit and 10 units at the upper limit. Therefore the new lower limit will be 50-10 = 40 units. The new upper limit will be 150 + 10 = 160 units.

- ❖ **Important:** Exercise caution when using extrapolation (Factor >1), as the implied assumption is that the relationship of the x and y variables is valid outside the range defined by the standards. This type of extrapolation should only be applied when prior knowledge of the relationship is known. Typically this feature is used in assays where linear relationships are known. **Inappropriate use of the Extrapolation Factor may invalidate results generated from data falling into the extrapolation zone.**

❖ **FYI:** Gen5 calculates the values that fall into the new limits. Those that fall outside these limits will be assigned $>$ than or $<$ than, respectively. Negative concentrations shall not be reported for any (y) value through extrapolation. If a value intersects the curve below 0 on the x-axis, Gen5 shall report a concentration of <0 for the well.

Advanced Parameter Constraints

 Gen5 lets you apply one of two types of constraint on your curve parameters to produce a more informative curve. (Detailed information about parameters is provided below.)

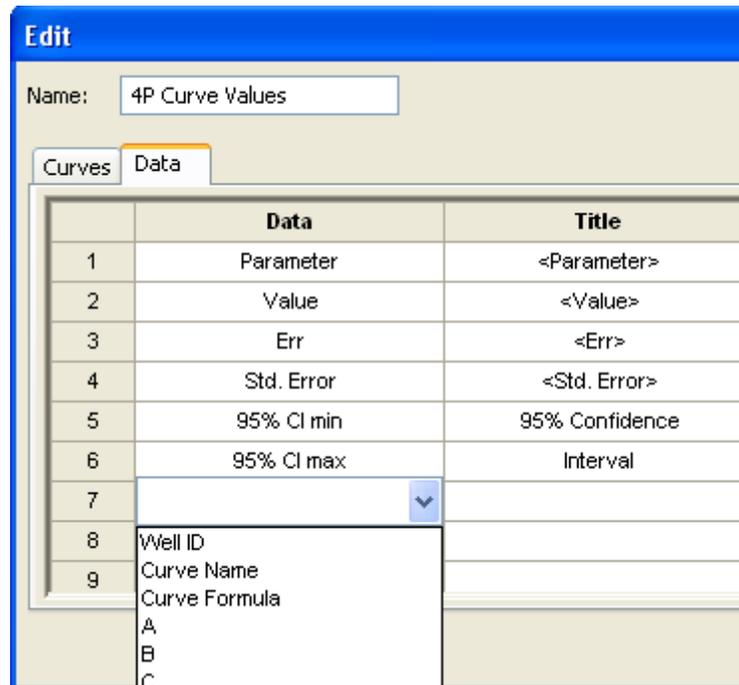
Select a constraint for a parameter:

1. Click in the **Mode** field of a parameter to enable a drop-down list to select:
 - **Start from:** is offered for the nonlinear regressions that use an iterative calculation process to determine the best fit. For 4P and 5P curve fits you can tell Gen5 to start the iteration process with a given value.
 - **Fixed:** lets you assign a fixed parameter value for Gen5 to use in the calculation. When you fix certain parameter values Gen5 will use them to determine the best-fit values for the other parameters.
 - **Estimate:** does not apply a constraint. It lets the curve fit determine the parameter value.
2. Enter a **Start From** or **Fixed** value in the **Value** field when applicable.

Helpful Information

- Constraints can be useful when you have not collected sufficient data to map all the parameters in your model and you know or expect a parameter to equal a certain value.
- 4P and 5P regressions require that B, C, and E parameters are positive (non-negative) integers. Gen5 prevents input of a negative or null value for these parameters with an error message.
- For Linear and Polynomial Regressions, fixing the B or A parameters (respectively) to 0 is similar to setting the [Y Axis Intercept](#) to 0
- Constraints can be added or modified at any time, before or after the data has been collected. When the regression calculation using initial, non-constrained values generates a curve that is far from the data, you may be able to generate a better fit using a Start From or Fixed constraint.

About Curve Parameters and Values



Depending on the curve fit method, Gen5 calculates and reports the values for these parameters. They can also be used in a Validation formula to test the results of your experiment.

Fitting Parameters

The coefficients used in the equation to calculate the curve are the fitting parameters, potentially: A, B, C, D, E, F, G. These values can be constrained or determined by the curve fit. Gen5 reports SE and 95% CI for each parameter, except when Parameter Constraints have given them fixed values.

For example, A, B, C, D are the 4 parameters in the 4P curve formula:

$$y = \frac{a-d}{(1+(x/c)^b)} + d$$

Goodness of Fit

R² - Coefficient of Determination

The value R² quantifies goodness of fit. The coefficient of determination of the regression ranges from 0.0 -1.0. It is computed by comparing the sum-of-the-squares distances from the best-fit curve and from a model defined by the null hypothesis, e.g. horizontal line through the origin.

- When R² = 0.0 the curve does not come close to the data. Knowing X does not help you predict Y.
- When R² = 1.0 all points lie exactly on the curve with no scatter. If you know X you can calculate Y exactly.

R^2 is one criterion for determining if your curve fit is reasonable. Review the other Curve Data, like confidence intervals, to confirm the value of a high R^2 .

Standard Error (SE)

Gen5 constructs a "Hessian Matrix", evaluates the WSS and DoF to calculate the asymptotic Standard Error of Estimate of each parameter. A low Standard Error indicates one or more of these conditions:

- the curve fit models the data well
- lots of data points
- less scattered data
- narrow confidence intervals

❖ SE is not reported for fixed parameters.

95% Confidence Interval: Min and Max

Review the 95% CI range for each parameter to evaluate the curve's goodness-of-fit. A narrow range assures you of a true best fit, but a wide range signals a weakness in the experiment or in the model selected to fit the data.

If you plotted the minimum and maximum CI values that Gen5 reports for each parameter it would produce a confidence band on equal sides of the curve. This standard statistics calculation tells you, with 95% certainty, the best-fit curve falls within the confidence band.

See [Calculating the Confidence Interval using t-distribution](#) on page 266.

❖ SE and 95% CI are not reported for fixed parameters.

General Metrics

R - Correlation Coefficient

Gen5 calculates R by taking the square root of the **coefficient of determination: R^2** .

The correlation coefficient ranges from -1 to 1. A value of 1 shows that a linear equation describes the relationship perfectly and positively, with all data points lying on the same line and with Y increasing with X. A score of -1 shows that all data points lie on a single line but that Y increases as X decreases. A value of 0 shows that a linear model is inappropriate – that there is no linear relationship between the variables.

Err - Error

ERR, also known as the "root mean squared error" (RMSE), is the difference between the actual measurements and the values predicted by the model. It can be used to determine whether the model fits the data or not.

SS — Sum of Squares

The sum of the squares of the vertical distances of the points from the curve. It is useful when comparing curve fits. The less scattered the data the smaller the SS and Std Err. When weighting is applied to minimize the relative distance squared, the WSS is used to assess goodness-of-fit.

Covariance Metrics

Degrees of freedom (DF)

$$DF = p - n$$

where **p** is the number of data points

and **n** is the number of parameters

t-Distribution (T)

t-distribution is a statistic whose values are given by:

$$t = [x - \mu] / [s / \text{sqrt}(n)]$$

where x is the sample mean, μ is the population mean, s is the standard deviation of the sample, n is the sample size, and t is the t score.

See [Calculating the Confidence Interval using t-distribution](#) on page 266.

Weighted Sum of Squares (WSS)

When you apply a weighting factor to the curve, Gen5 calculates the weighted sum of squares (WSS), then uses it to determine the SE for a parameter a_k :

$$SE(a_k) = \sqrt{\frac{w_i \times \sum_{i=0}^{n\phi} (y_i - f_X(x_i))^2 \times Var(a_k)}{n - p}} = \sqrt{\frac{WSS \times Var(a_k)}{DoF}}$$

where w_i is the weight of (x_i, y_i)

and p is the number of data points

and n is the number of parameters

Interpolations

Gen5 reports the user-defined interpolations **Y Formula** and the values it produces for **X** and **Y**.

Calculating the 95% Confidence Interval using t-Distribution

The 95% Confidence interval for a parameter a_k is calculated with the following formula:

$$\left[a_k - t_{0.025,df} \times SE(a_k); a_k + t_{0.025,df} \times SE(a_k) \right]$$

Where $t_{0.025,df}$ is the 97.5th percentile of the Student t distribution, given in the following Upper Tail Probability table:

Degrees of Freedom	<u>Pr(T > t)</u>	Degrees of Freedom	<u>Pr(T > t)</u>
1	12.706	31	2.040
2	4.303	32	2.037
3	3.182	33	2.035
4	2.776	34	2.032
5	2.571	35	2.030
6	2.447	36	2.028
7	2.365	37	2.026
8	2.306	38	2.024
9	2.262	39	2.023
10	2.228	40	2.021
11	2.201	41	2.020
12	2.179	42	2.018
13	2.160	43	2.017
14	2.145	44	2.015
15	2.131	45	2.014
16	2.120	46	2.013
17	2.110	47	2.012
18	2.101	48	2.011
19	2.093	49	2.010
20	2.086	50 to 59	2.009
21	2.080	60 to 69	2.000
22	2.074	70 to 79	1.994
23	2.069	80 to 89	1.990
24	2.064	90 to 99	1.987
25	2.060	100 to 119	1.984

Degrees of Freedom	Pr(T > t)	Degrees of Freedom	Pr(T > t)
26	2.056	120 to 139	1.980
27	2.052	140 to 179	1.977
28	2.048	180 to 199	1.973
29	2.045	200 to 499	1.972
30	2.042	500 to 999	1.965
		1000 and greater	1.962

Data In for Curve Analysis

Data Reduction > Curve Analysis > Data In tab

Curve Analysis

Curve Name: Give the curve a unique name

Data In | Curve Fit | Data Out

Generate Curve(s) from Current Plate

Well ID: Standard

X Axis Data:

Y Axis Data:

Use Curve from Other Plate

Curve Name:

OK Cancel

Generate Curve from Current Plate

A prerequisite for this option is defining in the [Plate Layout](#):

- Standards, Controls, or Dilution Samples and
- **Concentrations** or **Dilutions** for the standards, controls or samples

In each of the three fields, use the drop-down options to select the parameters for the curve:

- **Well ID** is the Standards, Controls or Dilution Samples to plot on the X axis
- for SPL (samples) and SPLC (sample controls), select All or one ID (see page 273)
- **X Data** is the source for the values to plot on the X-axis, by default it is set to use the map of the selected Well IDs <Plate Layout>
- **Y Data** is the source for the values to plot on the Y-axis

Use Curve from Calibrator Plate

This option only applies to **Other Plates** in a [Calibrator Plate Protocol](#) described in the **Multi-Plate Protocol** chapter

Data Out for Curve Analysis

Data Reduction > Curve Analysis > Data Out tab

The data sets created here will be available for viewing, reporting, and exporting. You may also be able to use them to perform additional data reductions.

Curve Analysis

Curve Name:

Data In | Curve Fit | Data Out

Change these default names for the generated output.

Concentrations

Calculate Concentrations

Data Set Name:

Calculate Concentration x Dilution

Data Set Name:

Unique output names are required when you're creating more than one curve.

Interpolations

	Formula
1	2.15
2	H12
3	STD5
4	CTL2*2
5	
6	Enter a value or formula to view and report that Interpolation result
7	
8	
9	
10	
11	

OK Cancel

Options Grayed Out?

Data Out options are only available when the curve is plotted for standards, controls or samples with **Concentration** values (not Dilution values).

For Calibrator-Plate Protocols Only

- ▼ **Y-Data:** Select the data set to use for plotting the curve, based on the standard curve from the Calibrator Plate.

Concentrations

Gen5™ lets you define multiple curves for an experiment or protocol, the only requirement is defining a unique name for each one in the **Data Set Name** and **Curve Name** fields.

Calculate Concentration x Dilution

If sample dilutions have been defined for this protocol in the Plate Layout, you can use this feature to perform the common Titer Curve requirement of calculating the actual concentration of diluted samples. You must define a unique name, **Data Set Name**, for each data output generated based on a different curve.

Interpolations

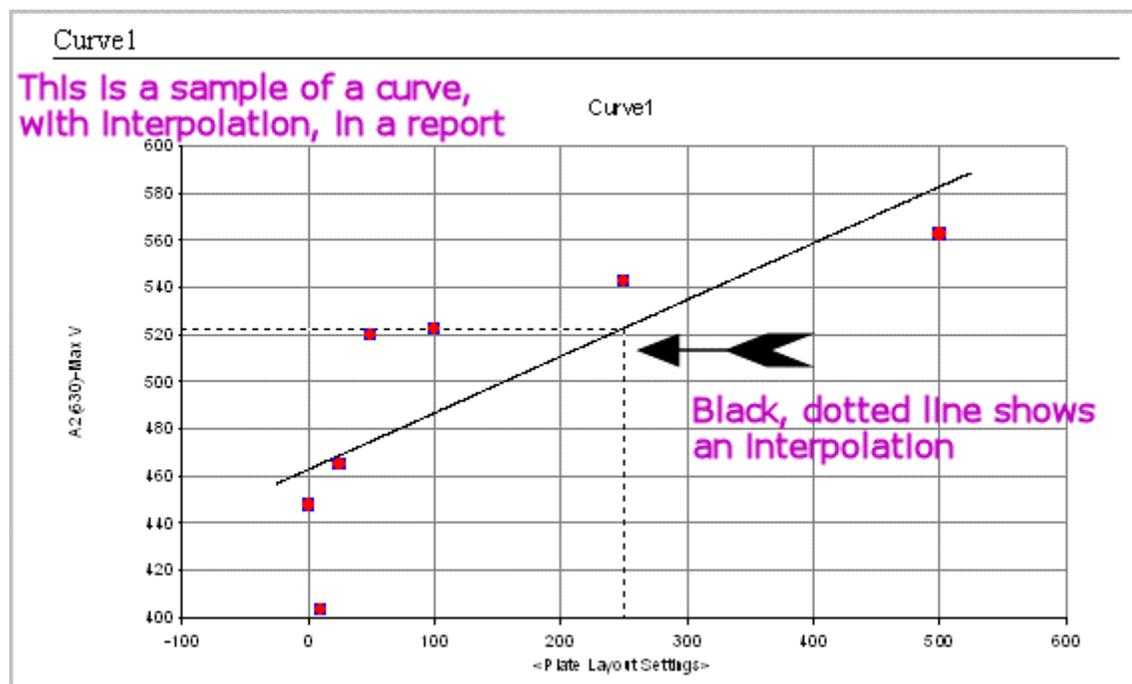
In the Interpolations table, you can enter up to 20 Y-Axis values or formulas to view and report the results. Well IDs (that have been previously) defined in the Plate Layout can be used. Review the Formula Syntax for Interpolations. Gen5 plots the interpolations in the generated curve if possible, Example.

Interpolation Formula Syntax

Symbol/Function	Definition	Example
-	Subtraction or negation	BLK-0.010
/	Division	CTL1/CTL2
*	Multiplication	STD1*0.10
()	Represents inclusion	(CTL1/CTL2)*100
Numeric value	Represents y value	0.500, 54000
Well coordinates	The location of a specific well	A1/B1
Well ID Well_All	The value of a specific well. The ID assigned to a specific well, including a Conc/Dil index value, if applicable: <ID><index>:<Conc/Dil Index> The last number is the Conc/Dil <u>index</u> , not the concentration/dilution value. The well index can be replaced by _ALL for samples and sample controls, which returns the mean of all indexes of the well type.	STD3 CTL1:2 SPL_ALL SPLC_ALL:3

MEAN(<ID>) Mean(x;y;z;...)	The mean of the specified well identifier, well coordinates or variables	MEAN(POS1) MEAN(A1;A2)
SD(<ID>) SD(x;y;z;...)	The standard deviation of the specified well identifier or variables	SD(BLK) SD(22;33;44)
CV(<ID>) CV(x;y;z;...)	The coefficient of variation of the specified well identifier or variables	CV(STD1) CV(A1;SPL3;50)
Round(x;y)	Rounds x to the y number of significant digits	Round(SPL3;4)
Truncate(x;y)	Truncates x to the y number of significant digits	Truncate(STD2;5)
Dil(<ID>) or Conc(<ID>)	Returns the defined dilution or concentration of the specified well ID	DIL(SPL1) Conc(CTL2)
LOG(x)	Represents the LOG10 function	LOG(SPL10)
POW(x;y)	The value of x raised to the power of y	POW(STD1;3)
SQRT(x)	The square root of <x>	SQRT(A1*B1)
MIN(x;y;z;...)	The minimum of the defined variables	MIN(CV(CTL1);CV(CTL2))
MAX(x;y;z;...)	The maximum of the defined variables	MAX(A1;B1;C1)
Data reduction variable	Represents a value collected with the Runtime Prompts	!Lot#
Functions allow a combination of expressions	(x;y) Any expression that represents a single value, including well identifiers, locations, numerals, a function that results in a single value, can be included in the formula, if it's a valid expression. Functions described with the ellipsis (x;y;z;...) allow up to 10 expressions.	

Interpolation Example



Interpolate the IC₅₀ or EC₅₀

When using the 4 Parameter or 5 Parameter curve fit methods, you can show the IC₅₀ or EC₅₀ value in the curve results using Gen5's Interpolate function.

1. First, define the Gen5 protocol and run the experiment as you normally would to capture the measurement results.
2. After Gen5 has calculated the best fit curve, locate and make note of the [Curve Parameters](#):
 - **4P**: C
 - **5P**: A and D. Then, calculate the IC₅₀/EC₅₀ value using this formula: $((A-D)/2)+D$
3. Return to and [edit](#) the **Data Reduction>Curve Analysis** step: select the Data Out tab and enter the value determined in the previous step in the **Interpolations** table under Formula.

In the Plate View select the Graphs tab to see the interpolated IC₅₀ or EC₅₀ value. You will also see the value plotted when you include the curve in a report or PowerExport.

Multiple Curves

Gen5's Multiple Curves Options

Gen5 offers several methods for generating multiple curves in an experiment. Follow the link for the method that best fits your needs:

- **Generating Multiple Standard Curves**: you have two or more standards and want to plot a standard curve for each
- **Generating Curves based on Sample Dilutions**: you have samples with multiple dilutions and want to plot curves based on the expected dilution values
- **Plotting Sample Dilutions on a Standard Curve**: you have unknown samples of various dilutions and want to determine their concentration from a standard curve
- **Kinetic curve for each well**: you are conducting a kinetic or time-course analysis and want to plot kinetic curves to determine Max V, Integral, etc.

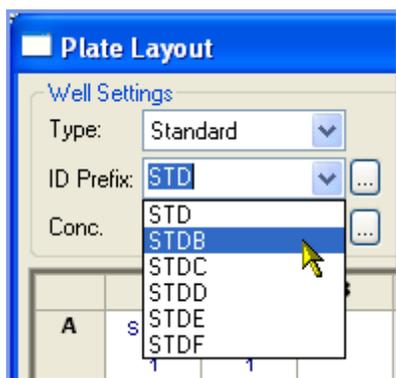
Generating Multiple Standard Curves

Gen5 lets you create up to six standard curves in an experiment. The standards can have the same or different concentration values.

How to:

Generating multiple standard curves requires applying multiple standards to the plate:

1. Create a new Protocol and define its Procedure in the usual way. When defining the Plate Layout, [assign the first set Standards](#) to the plate as usual



You can change the default IDs for the Standards, learn how in the **Preparing Plates** chapter

2. Use the **ID Prefix** drop-down list to select the next standard, e.g. STDB, to assign to the plate
3. Optionally, click the 3-dot button for **Conc.** to define different concentration values for the next standard. Overwrite the first set of concentration values,

with those for the current standard. Skip this step to use the same concentrations

4. Assign the location of the additional standards on the plate
5. Assign the location of the test samples and any controls to the plate
6. Go to **Data Reduction > Curve Analysis** to set up the curves. Create a Curve Analysis step, in the usual way, for each set of standards defined (one curve for each standard group).

To View Multiple Standard Curves:

- ▼ As with a single standard curve, after you've acquired the data, e.g. read the plate, open the Plate workspace, and select the **Graph** tab. Use the drop-down list in the **Curve** field to select the curve to view.

Curve Fitting Results: In addition to displaying the curve, Gen5 displays, in a table beneath the curve, the data points used to calculate all the standard curves.

Combine the Curves in One View: Gen5 lets you create a new curve to overlay multiple curves in one graph.

To Report Multiple Curves:

Gen5 lets you report/print the curves separately or combined:

- **Create a New Graph** to overlay curves and add the new curve to the **Content** in [Report Builder/Power Export Builder](#) or add each curve independently to report them separately
- For any curve, display it in the Graph tab of the Plate workspace and Right-Click to copy or save it as an image for use in a word-processor application
- [Export it to Excel®](#)

Samples and Sample Controls Curves

Data Reduction > Curve Analysis > Data In

Gen5 will plot a curve for each sample ID or sample control ID, using the expected dilutions/concentrations for the X-Axis and the user-selected measurement values for the Y-Axis. Titer assays, for example, use this feature to plot a titer curve.

Well ID

After setting the Well ID to SPL or SPLC (assuming the default IDs were retained), tell Gen5 to generate one curve for **All** (each of) the IDs for which a concentration or dilution has been defined, or for only one particular ID:

- Keep the check mark for **All IDs** to generate one curve for each ID
- Or, de-select All and enter the number of the Sample ID or Sample Control ID group for which you want only one curve plotted.

Viewing

Just like standard curves, after you've read the plate, open the Plate View and select the **Graph** tab. Use the **Curve** drop-down list to select the curve to display. Gen5 appends the Well ID (e.g. SPL1) group name to the curve name: Curve_SPL1 for example, to name the curves.

Reporting

You can [report/print the curves](#) just like standard curves, repeating the procedure for each curve generated, as needed.

❖ If the Samples or Sample Controls used to plot the curves are assigned Concentrations, rather than Dilutions, you can elect to **Calculate Concentrations** and **Concentrations x Dilutions**. When the Well ID to plot the curves is set to **All IDs**, the results will only be calculated for the samples used in generating each single curve. For example, concentrations for SPL1 will be calculated using the SPL1 curve, concentrations for SPL2 will be calculated using the SPL2 curve, and so on.

How to generate Sample-Dilution Curves

When you have multiple dilutions of your samples, Gen5 makes it easy to plot a curve for each sample based on the known dilution values. The critical factor is assigning the dilution values to the Samples in the **Plate Layout**. Gen5 recognizes sample dilutions as valid X-axis values.

How to:

1. Select **File>New Protocol**
2. Select **Protocol>Procedure** and define the **Read** step (and any other required steps)
3. Select **Protocol>Plate Layout**:
 - Define the **Sample Dilutions**
 - Assign the location of the sample dilutions, and blanks (if any) on the plate

For example:

Plate Layout						
Well Settings						Replicates
Type:	Blank					Number:
ID:	BLK					<input checked="" type="radio"/> Horz.
	1	2	3	4	5	6
A	SMP1:1 10	SMP1:1 10	SMP2:1 10	SMP2:1 10	SMP3:1 10	SMP3:1 10
B	SMP1:2 20	SMP1:2 20	SMP2:2 20	SMP2:2 20	SMP3:2 20	SMP3:2 20
C	SMP1:3 30	SMP1:3 30	SMP2:3 30	SMP2:3 30	SMP3:3 30	SMP3:3 30
D	SMP1:4 40	SMP1:4 40	SMP2:4 40	SMP2:4 40	SMP3:4 40	SMP3:4 40
E	SMP1:5 50	SMP1:5 50	SMP2:5 50	SMP2:5 50	SMP3:5 50	SMP3:5 50
F	SMP1:6 60	SMP1:6 60	SMP2:6 60	SMP2:6 60	SMP3:6 60	SMP3:6 60
G	CTL1	CTL1	CTL2	CTL2	CTL3	CTL3
H	BLK	BLK				

This example shows three samples with the same dilution values. Gen5 will create three curves, one for each sample. Gen5 assigns a dilution index to keep track of the multiple instances of a sample, e.g. SMP2:3 is the third dilution index of Sample 2.

4. Select **Protocol>Data Reduction**

- When Blanks (BLK) are assigned in the Plate Layout, Gen5 automatically creates the [Blank-Subtraction](#) transformation
- Depending on your assay requirements you may need to define another transformation or data reduction to normalize the data used in the curves, as in this [Toxicity/Cytotoxicity](#) assay

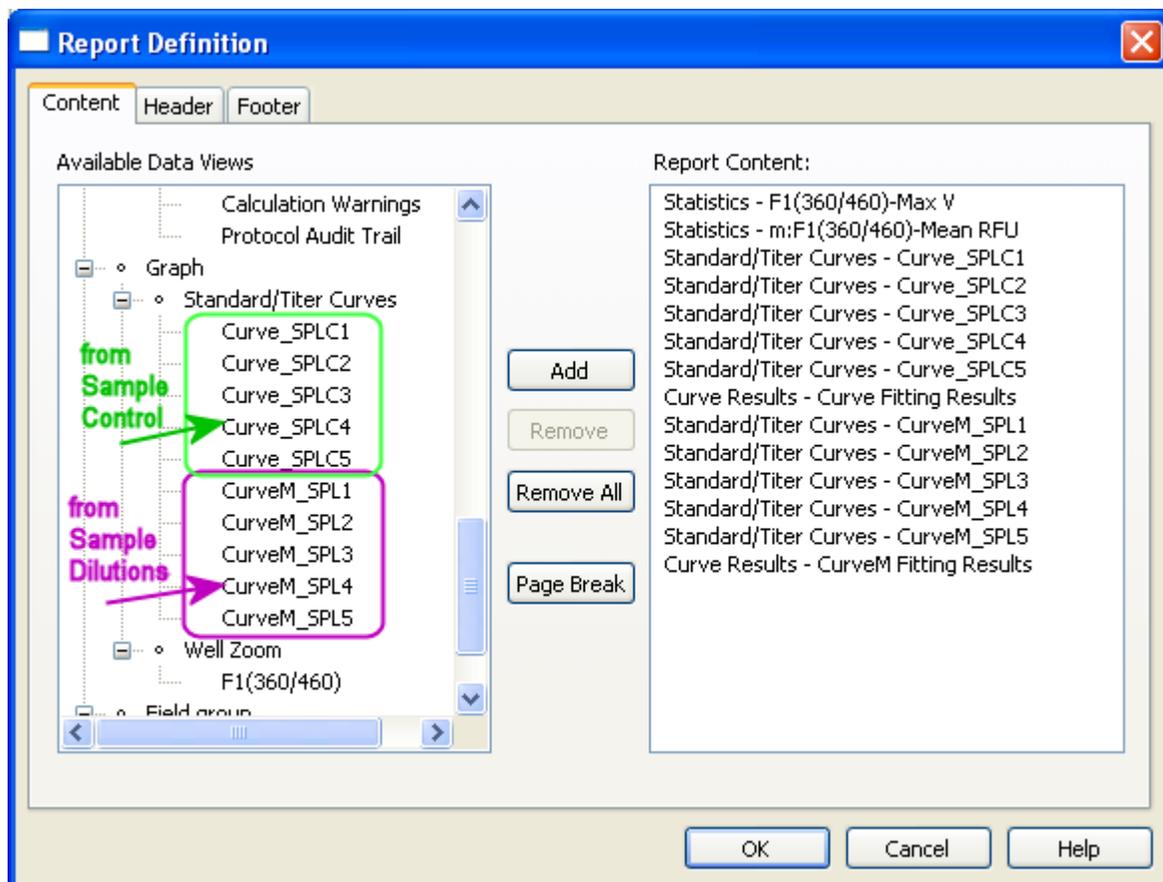
5. Click **Curve Analysis**

- On the **Data In** tab, set the **Well ID** to SPL (or the customized Well ID) for sample dilutions
- Select the **Curve Fit** tab to select the best curve fit method

Viewing and Reporting Sample Dilution Curves

You can overlay multiple sample dilution curves in one graph for viewing and reporting. After defining the Procedure, Plate Layout, and Data Reduction steps, **Create a New Graph in Data Views**. The data views become Available Data Views in:

- [Report Builder](#) to generate a print out
- **Power Export Builder** for transfer to Excel®
- To view the new curve online, select the **Graph** tab and use the **Curve:** drop-down list to select it



Plotting Sample Dilutions on a Standard Curve

When you have unknown samples and want to determine their concentration, it may be most efficient to test them in various dilutions/concentrations against a standard. In Gen5, you can take advantage of multiple features to accomplish this task.

How to:

1. Select **File>New Protocol**
2. Select **Protocol>Procedure** and define the **Read** step (and any other required steps)
3. Select **Protocol>Plate Layout:**
 - Define the **Sample Dilutions**
 - Define the **Standards** and their expected concentrations
 - Assign the location of the sample dilutions, standards and blanks (if any) on the plate
4. Select **Data Reduction> Curve Analysis** to create the standard curve
 - Gen5 may have generated a "corrected" data set: if you assigned blanks to the plate or selected Pathlength Correction in the Read step, you'll want to select these data sets for **Data In** for the **Y-Axis Data** when plotting the curve
5.  On the **Data In** tab, set the **Well ID** to **STD** and use the drop-down to select the **Y-Axis Data**
6. On the **Curve Fit** tab, choose a curve fit method
7. On the **Data Out** tab, define Interpolations to plot select Sample dilutions on the curve. For example, in the **Interpolations Formula** table enter SPL1:1, SPL1:5, SPL2:1, etc. to easily identify them in the graph

 **Viewing results:** Select the **Graphs** tab, after reading the plate, to view the curve. Use the drop-down for the **Results** field (at the top, right corner) to select the Interpolations table to enhance the view

Troubleshooting Curve Fits

Gen5 gives you a lot of flexibility in curve fitting, some of them can be used to improve a curve fit:

- **Collect more data:** the most certain way to improve a curve fit is to collect more data: widen the range of X values, for example.
- **Simplify the model:** Linear regression and 4P fits are easier to calculate and have fewer unknowns than a 5P fit, your data may better fit a simpler model. (You can also use constraints to simplify the model, i.e. reduce the number of unknowns.)
- **Apply constraints** when the curve is far from the data: by constraining the initial values (Start From) or assigning a Fixed value you may be able to generate a better fit.
- Constraints can also be useful when you have not collected sufficient data to map all the parameters in your model and you know or expect a parameter to equal a certain value.

 Gen5 lets you modify the settings used to plot the curve, as suggested above, so you can experiment with the different options. Alternatively, you can create multiple curves using the same data but with variant settings (add more Curve Analysis steps to the Data Reduction), so it is easier to compare the various results. Then, you can overlay the curves in one view, if that is helpful: see [Create a New Graph](#).

Kinetic Analysis Options

❖ Learn more about the Well Zoom in the **Kinetic Analysis** chapter.

Gen5 offers a full complement of options for analyzing your [Kinetic Reading](#) raw data results.

Data Reduction Options

In the **Data Reduction > Well Analysis** dialog, you can set up multiple steps, taking advantage of some or all the available options:

- Mean V
- **Max V** (Gen5 creates this Data Reduction step automatically. It also calculates the Y Intercept, R & R², time at Max V, and Lag time)
- Mean Min/Max OD/RFU/RLU
- Onset OD/RFU/RLU
- Onset OD%/RFU%/RLU%
- Integral

Combine Data Sets

Gen5 lets you combine the measurements collected from multiple kinetic readings performed in one experiment. When multiple kinetic loops are defined in the **Procedure**, Gen5 offers the **Append to previous Kinetic data** option in the second and subsequent kinetic read steps.

Append to previous Kinetic data

The read steps in the kinetic loops must be identical, and Gen5 enforces this rule when the **Append** option is selected. In a **Synchronized Mode** block, this feature is mandatory and Gen5 automatically applies it to the second and higher kinetic read steps.

Append to previous Kinetic data results in one data set containing all the measurement values acquired from the reader during the all the kinetic loops. Do not use this option if you want separate data sets for data reduction purposes.

Dispensing Reagent in a Kinetic Analysis

Gen5 lets you include dispensing in a kinetic analysis: learn about [Dispensing Reagent in a Kinetic Analysis Protocol](#) in Gen5's Help.

Perform Well Analysis

Data Reduction > Well Analysis

About Gen5's Well Analysis

- Well Analysis is available as a Data Reduction option when a [Kinetic](#) analysis has been set up in the **Procedure**
- Well Analysis is also available when **Spectrum**, **Area Scan**, or **Linear Scan** is defined as the Read Type
- Well Analysis produces a special data view that lets you zero-in on each individual well to see the measurement values and analysis results: **Well Zoom**
- Well Analysis also produces a [special table](#) of results for viewing, reporting and exporting the calculation results of each well
- In the **StepWise Data Reduction** dialog, you can create multiple Well Analysis events to obtain multiple types of results. Assign a unique Label/name to each well analysis step.

How to:

1. **Label** - Identifies the results for data views, report building, etc. Enter a unique name/ID to be used in the resulting data set name. Data Set Name Rules. You must enter a unique Label when a data set is used in more than one Data Reduction step.
2. **Data In** - Click the down arrow to select a data set, e.g. raw data or results of previously-defined Transformation (based on All Reads)
3.  **Calculation Type** - Use the buttons to select the type of computation to conduct. Each option displays the results it will produce in the **Generated Data** box on the right side of the screen (see below)
 - **Calculation Options** correspond to the Calculation Type selected. Click the Calculation Options button to alter the default settings for the selected **Calculation Type**.
 - [Formula Syntax for Well Analysis](#): describes the symbols and functions supported to perform a calculation using the individual read points of each well (on page 282).

Kinetic and Scanning Data Reduction Outputs Listing

Set up the [Well Analysis](#) option that generates the desired data output

Read Type	Calculation Type	New Data Sets
Kinetic	Mean V	Mean V R (correlation coefficient) R ² (coefficient of determination) Y Intercept
	Max V	Max V R (correlation coefficient) R ² (coefficient of determination) t ⁽¹⁾ at Max V Lagtime Max V Calc - t min Max V Calc - t max Y Intercept
	Mean Min/Max OD	Mean Min OD ^[2] t at Mean Min OD Mean Max OD t at Mean Max OD
	Mean, Std, CV	Mean OD Std Dev OD CV OD
	Onset OD	Onset Time Onset OD Basis Time Basis OD
	Integral	Integral
Spectrum	Min / Max OD	Mean Min OD W ^[3] at Mean Min OD Mean Max OD W at Mean Max OD

Read Type	Calculation Type	New Data Sets
Linear Scan	Mean, Std, CV	Mean OD Std Dev OD CV OD
	Mean Min/Max	Mean Min OD Read Pos ^[4] at Mean Min OD Mean Max OD Read Pos at Mean Max OD
	Integral	Integral
Area Scan	Mean, Std, CV	Mean OD Std Dev OD CV OD

OD assumes absorbance reads. Substitute RFU or RLU/sec as appropriate.

[3] W = Wavelength

[4] Pos = Horizontal Reading Position

Formula Syntax for Well Analysis

The [Well Analysis Formula](#) lets you perform calculations using the individual read points or indexes in "multi-index" readings: kinetic and spectral scan. Formulas can be written similar to Transformations, using the symbols and functions listed below. Also see some [Examples](#) on page 285.

❖ Symbols and functions are not case sensitive.

Symbol/Function	Description	Example
R#	Read point or index of a kinetic read	R1
W#	Read point or index of spectral read	W2
Function Operators	Add +, subtract -, divide /, multiply *, combine ()	R2+R4, R6-R10, W3/W8, R1*R15, (R4/R1)*100
Numeric value	Any numeral, including those expressed with scientific notation	2.45E-08
Mean(R#/W#;...) Mean(x;y;z;...)	The mean of a set of read points or variables	Mean(R2;R4;R8) Mean(1;2;3)
SD(R#/W#;...) SD(x;y;z;...)	The standard deviation of specified read points or a set of numerals	SD(R12;R22) SD(23;75;45)
CV(R#/W#;...) CV(x;y;z;...)	The coefficient of variation of specified read points or any set of numerals, expressed as a percent	CV(W5;W6;W7) CV(1;2;3)
Round(x;y)	Rounds x to the y number of significant digits.	Round(W2;5)
Truncate(x;y)	Truncates x to the y number of significant digits.	Truncate(R3;3)
Log(x)	Represents the Log10 function	Log(R3)
POW(x;y)	Represents the value of x raised to the power of y	POW(W10;2)
POW(10;x)	Calculates the Anti-Log of the current well	POW(10;R4)
SQRT(x)	Represents the square root of x	SQRT(R1*R5)
MIN(x;y;z;...)	Returns the minimum of the defined variables.	Min(R5;R6;R7;R8)
MAX(x;y;z;...)	Returns the maximum of the defined variables	MAX(W1;W2;W3)

Symbol/Function	Description	Example
Functions allow a combination of expressions	(x;y) Any expression that represents a single value, including well identifiers, locations, numerals, a function that results in a single value, can be included in the formula, if it's a valid expression. Functions described with the ellipsis (x;y;z;...) allow up to 10 expressions.	

Using individual Kinetic read points in a Formula

In addition to Gen5's [Transformation](#) option, you can perform calculations using the individual reading points of a well in a Kinetic or Spectral analysis with the **Well Analysis Formula** function. The formula will generate a single value for each well. Gen5 names the resulting data set "Formula Result [nm]", where *nm* is the wavelength or filter set defined in the read step. When a Label is defined it precedes the naming convention: "Label: Formula Result [nm]"

After setting up the Kinetic loop or spectrum read step in the Procedure:

1. Select **Data Reduction>Well Analysis**
2. Select the multi-index data set to use for **Data In**
3.  Select the **Formula** button and write the calculation in the text field

Examples:

1. For an **ORAC Antioxidant Test**:

$$0.5+(R2/R1)+(R3/R1)+(R4/R1)+(R5/R1)....+(Rn/R1)$$

Where R1 is the reading at initiation of the reaction and Rn is the last measurement. A step-by-step description for using this formula in an experiment is described in the [Kinetic Fluorescence Assay Example](#) in the Assay Examples chapter.

2. To determine the mean of certain read points: $(r1xr2xr3xr4)/4$

Viewing/Reporting Results

Gen5 automatically generates a Matrix view and two tables when a Well Analysis Formula is created. In [Data Views](#), and the [Report Builder](#) and export options, you'll find:

- Matrix: Formula Result [*nm*]
- Statistics: Formula Result [*nm*]
- Well Analysis Results: Formula Result [*nm*]

Well Zoom during a Kinetic Read

Learn more about the Well Zoom in the **Kinetic Analysis** chapter.

1. Select the **Curves** data set from the **Data** drop-down list before **Reading** the plate.
2. **Zoom into a specific well**: click on the desired well to display the Well Zoom screen. It will display the results as they're measured.

 **Tip:** If necessary, drag the progress dialog out of the way for an unobstructed view: click and drag the title bar of the dialog to move it out of the way.

 **Caution:** In some cases, displaying the **Curves** data set during a Kinetic read can consume excessive resources resulting in computer-performance degradation. Learn more in the **Troubleshooting** chapter. You can follow the steps above to monitor the progress of one well, then, leaving the Well Zoom open, change the Matrix Data to a numeric view

Define Cutoffs

Protocol > Data Reduction > Cutoffs

About Cutoffs

Gen5™ lets you define cutoffs as fixed or variable margins against which results are compared. Gen5 creates a **Symbols** data set based on your definition of cutoffs that shows how each well fits into the defined categories. Gen5 also adds another tab to the **Plate View** to show the Cutoff Values, the results of cutoff formulas. Up to 40 formulas (of increasing value) can be defined for each data set and multiple Cutoff steps, one Cutoff step per data set, can be defined in the StepWise Data Reduction.

In the Cutoffs dialog, notice that the number of Symbols fields exceeds the number of cutoff formula fields by one. During data reduction, if the value of a well is less than the first cutoff, the first symbol is applied to it. If the value of a well is greater than or equal to the first cutoff but less than the second cutoff, the second symbol is assigned to the well, and so on.

Out-of-Range Symbols are applied to concentration values (calculated from a standard curve) when the concentrations are outside the extrapolation range of the curve: below the minimum or above the maximum. You can change the OUT+ and OUT- labels applied to these values by clicking in the text fields and replacing the content.

Data In data sets presented for selection are determined by the current protocol excluding multi-index data sets. Cutoffs cannot be determined in multiple-read measurements like kinetic analysis and scanning results. For these types of analysis a transformed or processed data set must be used.

????? — This symbol in Cutoff results, i.e. the **Symbols** data set, indicates an out-of-range or biased value was used in the calculation. Check the **Protocol Options > Calculation Options** settings.

❖ See an example and explanation of cutoffs on page 288

How to set up cutoffs:

1.  Begin by selecting the **Data In** data set on which to apply the cutoffs.

❖ **Hint:** The **Data In** drop-down list offers the data sets resulting from all previously defined reads (raw data) and calculations, except for multi-index readings, e.g. kinetic. Cutoffs cannot be evaluated against multiple-index reads.

2. Define the cutoffs, beginning with the lowest value, by entering a formula or data point in the **Cutoff Formula 1** field. In the **Symbols** fields that straddle the cutoff formula field, in the top field, enter a symbol (or call) to apply to wells with values less than the formula. In the next Symbol field, enter the symbol to

apply to values greater than or equal to the Formula. (See the Formula Syntax table on page 288.)

❖ **Important:** Cutoffs must be defined in increasing order, from the lowest value to the highest. Gen5 will issue a Calculation Warning and will be unable to assign Symbols to wells if the reading results do not affirm the increasing values of the cutoff formula

3.  Customize the appearance of the symbols, change the font and color, by clicking the 3-dot button next to the **Symbol** field.
4. Continue defining cutoffs and symbols in the same manner, always defining a higher value cutoff formula.

How to view the cutoffs:

1. After reading the plate (or otherwise acquiring data), open the Plate View and select the **Matrix** tab
2. At the **Data** field, use the drop-down list to select the **Symbols** data set
3. Select the **Cutoff Values** tab to view the values (formula results) upon which the Symbols data set was based

Important Information:

- When cutoffs, or calls, are defined, Gen5 adds a tab to the **Plate View** to display the values or results of the cutoff formulas
-  Up to 40 cutoff formulas can be defined for one data set. Use the scroll controls at the top of the dialog to display additional cutoff formula and symbol fields.
- [Multiple Cutoffs](#) can be defined (in the Data Reduction dialog) for a protocol: one set of cutoffs per data set, i.e. you can only reference a data set one time for cutoffs
- Gen5's rounding function in Data Views may cause unexpected results in the Cutoffs' reporting. Gen5 does not round the values before applying the cutoff formula, so it's possible to see conflicting outputs. More:

Cutoffs Example

Cutoff Dialog

Data In: Blank 490

Take Note
(Define from lowest value to highest value)

Cutoff Formula 1: NC

Cutoff Formula 2: PC

Cutoff Formula 3:

Cutoff Formula 4:

Symbols

NEG

EQV

POS

Out-of-Range Symbols

Values below range: OUT-

Values above range: OUT+

For this example, we customized the **Assay Control** well type in **Plate Layout** to put Positive (PC) and Negative (NC) controls on the plate. Then, setting up the cutoffs as shown in this image, Gen5 will apply **NEG** to any well with a value less than the NC. It will apply the **EQV** symbol to any wells that are greater than or equal to the NC but less than the PC. It will assign **POS** to any wells equal to or greater than the PC. Any wells with values considered out-of-range will be assigned OUT- if the concentration is below the minimum, and OUT+ if the concentration is higher than the maximum.

To view the cutoffs, after reading the plate, in the Plate workspace, select **Symbols** from the Data field drop-down list.

Formula Syntax for Cutoffs

Symbol/Function	Description	Example
+ - * /	Mathematical operators	CTL1+0.100
()	Represents inclusion	(CTL1/CTL2)*100
Numeric value	Any numeral, including those expressed with scientific notation	0.500, 54000, 1e10

Symbol/Function	Description	Example
Well ID Well coordinates wellID_ALL	<p>The value of a specific well. The ID assigned to a specific well, including a Conc/Dil index value, if applicable: <ID><index>:<Conc/Dil Index> The last number is the Conc/Dil <u>index</u>, not the concentration/dilution value.</p> <p>The well index can be replaced by _ALL for Samples and Sample Controls, which returns the mean of all indexes of the well type. Does not apply to STD, BLK or Assay Controls.</p>	A1 STD3 CTL1:2 SPL_ALL SPLC_ALL:3
MEAN(<ID>) Mean(x;y;z;...)	The mean of the specified well identifier or variables	MEAN(SPL1) Mean(A1;22;SPL2)
SD(<ID>) SD(x;y;z;...)	The standard deviation of the specified well identifier or variables	SD(BLK) SD(4;H12;CTL2)
CV(<ID>) CV(x;y;z;...)	The coefficient of variation of the specified well identifier or variables, expressed as percent	CV(STD1) CV(SPL10;33;B2)
DIL(<ID>); Conc(<ID>)	Returns the defined dilution or concentration of the specified well ID	Dil(CTL3) CONC(STD2)
Round(x;y)	Rounds x to the y number of significant digits. x can be any valid symbol or expression. (Learn more below)	Round(SPL1;4)
Truncate(x;y)	Truncates x to the y number of significant digits. x can be any valid symbol or expression	Truncate(CTL3;3)
LOG(x)	Represents the LOG10 function	LOG(SPL10)
POW(x;y)	The value of x raised to the power of y	Pow(STD1;3)
SQRT(x)	The square root of x	SQRT(A1*B1)
MIN(x;y;z;...)	The minimum of the defined values	Min(CV(CTL1); CV(CTL2); 45)
MAX(x;y;z;...)	The maximum of the defined values	MAX(A1; B1; C1)
Data reduction variable	Represents a value collected with the Runtime Prompts	!KitFactor

Symbol/Function	Description	Example
<p>Functions allow a combination of expressions</p>	<p>(x;y) Any expression that represents a single value, including well identifiers, locations, numerals, a function that results in a single value, can be included in the formula, if it's a valid expression. Functions described with the ellipsis (x;y;z;...) allow up to 10 expressions.</p>	

Rounding Issue with Cutoffs

It's possible to see apparent discrepancies in the cutoff results:

For Example: a value of .9998 will be rounded to 1 when the numeric format for the view is set to 3 Decimal places (the default setting). In the Matrix view you'll see 1 for the well, but if Cutoffs are set to identify values less than 1, it will be reported as <1 in the Symbols view/report.

Two ways to avoid this inconsistency:

- Create a **Transformation** that rounds the data set, and apply the cutoffs to the new data set. Transformation formula: **Round(X;3)** for example. The resulting reports and views of the data set and cutoffs will be consistent.
- Revise the numeric **Format**, i.e., the number of decimal places or significant digits, of the Data View to more precisely view and report the values. Find instructions at [Numeric Format](#). In the above example, increasing the number of decimal places to 4 would eliminate the inconsistency. This option may or may not be appropriate for your results.

Validation

Data Reduction > Validation

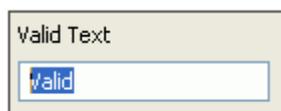
Many assay kits define conditions which must be met to validate the results of an experiment. The Validation dialog lets you set up these conditions to compare to selected results (data sets). The **Validation Formulas** can be fixed thresholds or algebraic formulas.

Top 5 Things to Know about Validation Criteria:

1. Multiple Validation steps can be defined in the StepWise Data Reduction, but only one set of criteria for each data set is permitted
2. Up to 50 conditions or formulas can be defined in a Validation step
3. When data points that cause a Validation failure are masked or changed, the Validation can change from fail to pass
4. Validation results can be included in reports and/or export content
5. Most often, validation formulas combine well IDs with a less than, greater than, or equal sign, e.g. $STD1 \geq 1.5$:
 - < less than
 - <= less than or equal to
 - > greater than
 - >= greater than or equal to

Setting up Validation Criteria

1. Select **Data Reduction > Validation**
2. Use the drop-down list to select a **Data In** data set against which the criteria are evaluated
3. In the **Formula** fields, enter conditions (up to 20) that must be met for the experiment's results to be considered valid.



4. Optionally, in the text fields on the right-side of the screen, e.g. Valid Text, click inside the field and overwrite the default text to change it for the results output and reports.

Formula Syntax for Validation

In conjunction with a **conditional operator**: <, <=, =, >, >=, you can use the following elements to define a formula as a validation or quality control condition:

Symbol/Function	Description	Example
+	Addition	CTL1+0.100<3.5
-	Subtraction or negation	BLK-0.010
/	Division	CTL1/CTL2
*	Multiplication	STD1*0.10
()	Represents inclusion	(CTL1/CTL2)*100
Numeric value	Any numeral, including those expressed with scientific notation	0.500, 54000, 2.45E-08
Well ID Well coordinates wellID_ALL	The value of a specific well. The ID and index assigned to a specific well. The well index can be replaced by _ALL for samples and sample controls, which returns the mean of all indexes of the well type.	A1 STD3 SPL_ALL SPLC_ALL: 3
WellID# WellID:# WellID#:#	To evaluate all of the specified wells individually, returns the mean value of each <u>separate</u> well index. The Conc/Dil index can also be represented with #. SPL#:# performs the evaluation on each dilution of each sample group.	STD# performs the evaluation on each STD group, e.g. STD, STDB, STDC, etc. SPLC2:# performs the evaluation on each SPLC2 dilution STD:# performs the evaluation on each concentration of the STD group, e.g. STD1, STD2
wellID:conc index	Use : as a separator to identify individual well IDs of a specific concentration or dilution, if applicable: <ID><index>:<Conc/Dil Index> The last number is the Conc/Dil <u>index</u> , not the concentration or dilution value.	CTL2:3 the average value for all CTL2 at the third concentration value

Symbol/Function	Description	Example
<p>The difference between the two "all" identifiers extends to their results output: using _ALL produces a one line result for all wells: valid or invalid (if one well is invalid, the report is invalid); the # produces individual invalid results: if one or more wells are invalid, each well result is reported; if all wells are valid a one line report is given. _All does not apply to STD, BLK or Assay Controls</p>		
SD(<ID>) SD(x;y;z...)	The standard deviation of the specified well identifier or expression	SD(BLK) SD(A1;STD3;45)
CV(<ID>) CV(x;y;z...)	The coefficient of variation of the specified well identifier or expression	CV(STD1) CV(SPL12;33;B2)
ROUND(x;y)	Rounds x to the y number of significant digits	Round(SPL1;4)
Truncate(x;y)	Truncates x to the y number of significant digits	TRUNCATE(STD1;5)
Conc(<ID>)	Returns the defined concentration of the specified well ID	CONC(CTL4)
DIL(<ID>)	Returns the defined dilution of the specified well ID	Dil(SPL3)
SQRT(x)	The square root of x	SQRT(A1*B1)
LOG(x)	Represents the LOG10 function	LOG(SPL10)
POW(x;y)	The value of x raised to the power of y	POW(STD1;3)
MIN(x;y;z;...)	The minimum of the defined variables	MIN(CV(CTL1);CV(CTL2))
MAX(x;y;z;...)	The maximum of the defined variables	MAX(A1;B1;C1)
Curve(<x>;<y>)	Returns the value of the selected curve result	Curve(CurveA;R2)>8.8
Curve(<x>;<y>;<z>)	Returns the value of the curve result for the selected parameter. See explanation below.	Curve(CurveB;C;SE)<=1
Data reduction variable	Represents a value collected with the Runtime Prompts	!KitFactor

Symbol/Function	Description	Example
Functions allow a combination of expressions	(x;y) Any expression that represents a single value, including well identifiers, locations, numerals, a function that results in a single value, can be included in the formula, if it's a valid expression. Functions described with the ellipsis (x;y;z;...) allow up to 10 expressions.	

Defining curve results and parameters

When you have generated a standard curve, Gen5 lets you define validation criteria using the [Parameters and Values](#) calculated by the curve as the variables in the validation formula. For example, you can define a validation condition specifying the value of R2:

$$0 < \text{Curve}(\text{CurveC};\text{R2}) < 1.0$$

Two types of curve formula are possible:

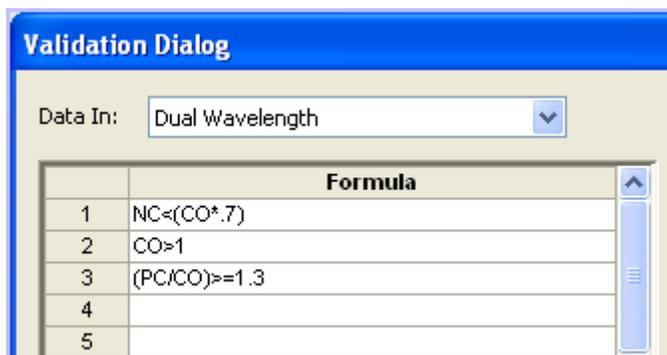
- Curve(Curve_name;y) to express a whole curve variable, such as R2, DF, or SS
- Curve(Curve_name;y;z) to identify a parameter and parameter-specific variable, like SE or 95% CI

❖ Note: you can select any data set in the experiment for this curve formula application; but, keep in mind Gen5's limitation of one Validation step per data set.

❖ Find more details in Gen5's Help. Look for Validation: Curve Formula

Validation Examples

1. From an assay kit: "The absorbance of the Negative Control (NC) must be less than 70% of the cutoff (CO) value. The mean absorbance of the cutoff should be greater than 1. The ratio Positive Control (PC)/CO should be greater than or equal to 1.3."



2. Another assay kit says: "Individual negative control (NC) absorbance values must be less than or equal to 0.150 and greater than or equal to -0.005. Individual positive control (PC) absorbance values must be greater than or

equal to 0.600. Individual positive control values must be within the range of 0.5 to 1.5 times the mean of the positive control."

Validation Dialog

Data In: Dual Wavelength

	Formula
1	$-0.005 \leq B1 \leq 0.150$
2	$-0.005 \leq C1 \leq 0.150$
3	$-0.005 \leq D1 \leq 0.150$
4	$E1 \geq 0.600$
5	$F1 \geq 0.600$
6	$0.5 * PC \leq E1$
7	$0.5 * PC \leq F1$
8	$E1 \leq PC * 1.5$
9	$F1 \leq PC * 1.5$
10	
11	

**In the plate layout:
NC in wells B1, C1, D1
PC in wells E1, F1**

- In a kinetic analysis, during the first of several readings, the mean value of STD1 must fall between 0.200 and 0.300 OD. The mean value of STD2 must fall between 0.900 and 1.300 OD.

Validation Dialog

Data In: A1(455) Read(s): 1

	Formula
1	$0.200 < STD1 < 0.300$
2	$.900 < STD2 < 1.3$
3	

Valid Text: Valid

And, the Mean OD of every sample replicate group must be greater than 0.

Validation

Data In: Mean OD: Mean Min OD [A1:455]

	Formula
1	$0 < SPL\#$
2	
3	

Viewing the Validation Results

Gen5 applies a status to each formula defined in the Validation step:

- **Valid** if the condition was met
- **Not Valid** if the condition was not met (i.e. failed).
- Unable to Evaluate, if:

- The selected data set was not available,
- A well ID in the formula was not found in the Plate Layout,
- A value could not be determined for a cited Well ID (e.g., all wells with that ID are masked, or over-ranged).

- ❖ You can change the status labels, replacing them with terms that have more meaning for your organization.
- ❖ Gen5 issues a Calculation Warning when out-of-range or biased values are used in a validation formula, see Calculation Options

- Gen5 adds a tab to the Plate View to display **Validation** results



- Failed validations or when Gen5 was "Unable to Evaluate" a criteria are reported immediately after a plate is read in the **Calculation Warning** pop-up. You can reopen the message anytime by selecting **Calculation Log** under the respective Plate in the menu tree. If the condition is **Verified**, Gen5 does not present a message.

Reporting Validation Results

-  Add the Validation table to Report Content in the **Report Builder**. Similarly, you can export the results.

Fluorescence Polarization

Data Reduction > Polarization

About Fluorescence Polarization Data Reduction

Gen5 automatically performs data reduction when Fluorescence Polarization (FP) is the selected read type. You can retain, modify, or delete the auto-generated data reduction step.

When Blanks are assigned to the Plate Layout, Gen5 performs a blank-subtraction before calculating polarization. When FP is performed in a kinetic loop, Gen5 uses the polarization results to automatically perform a Max V Well Analysis determination. Blank subtraction is performed before the Well Analysis, if applicable.

Polarization and Anisotropy

The basic polarization formulas use:

- **Parallel Intensity** ($I_{||}$): raw or blanked data from parallel measurement
- **Perpendicular Intensity** (I_{\perp}): raw/ blanked data from perpendicular measurement x G Factor
- **G Factor**: a coefficient used to calculate the polarization value. It corrects for the optical variations between the parallel and perpendicular emission paths unique to each reader. Gen5's default value is 0.87.

Gen5 automatically performs the Polarization calculation option. You can modify the automatically-generated FP data reduction step to change the output to Anisotropy, or to select both options.

The raw and transformed data sets created by FP are available for selection/use in all other applicable data reduction options, e.g. Transformations, Curve Analysis.

FP Formulas

$$P = \frac{\parallel - \perp}{\parallel + \perp} \quad \text{Basic Polarization}$$

$$r = \frac{\parallel - \perp}{\parallel + 2 \times \perp} \quad \text{Basic Anisotropy}$$

$$P = \frac{(\parallel(\text{spl}) - \parallel(\text{blk})) - (\perp(\text{spl}) - \perp(\text{blk}))}{(\parallel(\text{spl}) - \parallel(\text{blk})) + (\perp(\text{spl}) - \perp(\text{blk}))}$$

Blanked Polarization

$$r = \frac{(\parallel(\text{spl}) - \parallel(\text{blk})) - (\perp(\text{spl}) - \perp(\text{blk}))}{(\parallel(\text{spl}) - \parallel(\text{blk})) + 2 \times (\perp(\text{spl}) - \perp(\text{blk}))}$$

Blanked Anisotropy

- \parallel = **Parallel Intensity**: raw data from parallel measurement
- \perp = **Perpendicular Intensity**: raw data from perpendicular measurement X G Factor
- **G Factor**: coefficient used to correct for the optical variations between the parallel and perpendicular emission paths. Gen5's default value is 0.87
- spl = test sample; blk = blank

Gen5's Polarization formula

$$P = \frac{(\parallel(\text{spl}) - \parallel(\text{blk})) - G \times (\perp(\text{spl}) - \perp(\text{blk}))}{(\parallel(\text{spl}) - \parallel(\text{blk})) + G \times (\perp(\text{spl}) - \perp(\text{blk}))}$$

G = G Factor

The G Factor is used in the FP calculation to normalize the polarization value obtained on fluorescein to 20 mP (known reference value of unbound fluorescein). Gen5 ships with a "G Factor Determining Protocol" that you can run to determine your reader's specific G Factor for fluorescein.

Chapter 14

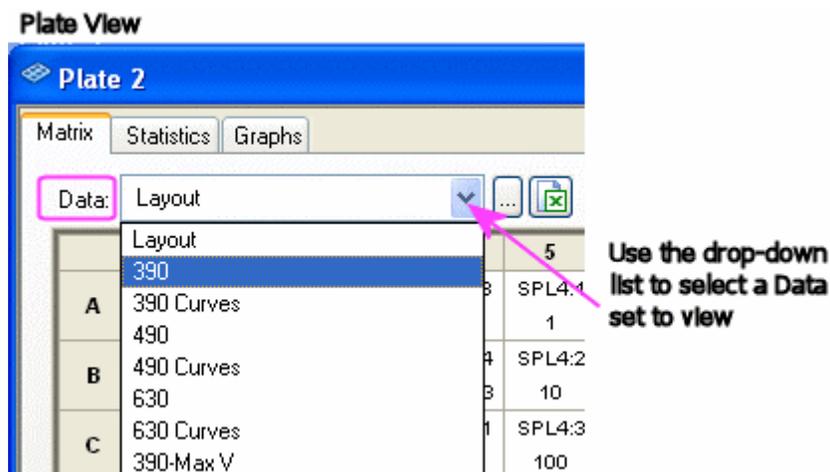
Viewing Results

Learn Gen5's naming conventions and the meaning of symbols and notations in this chapter. Instructions and suggestions for customizing online views and report outputs are provided also.

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Symbols and Notations	307
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Viewing Results

You can instantly view the results of an experiment in Gen5's main workspace using the **Plate View**:



- After reading the plate (or otherwise acquiring data), in the **Plate View** use the drop-down list for **Data** to display the raw data and any data reduction results
-  Click the **Quick Export** button to instantly open the current view in Excel®. Learn more about Gen5's Export Options
-  Click the 3-dot button next to a data set to customize the view's appearance. This feature is also available in the [Data Views](#) dialog.
- ** **Asterisks** are used to signal a change: in Gen5's title bar an asterisk indicates the current file has been changed but not-yet saved. When a data set is enclosed by asterisks it has become invalid. Generally this is because a Read step or Data Reduction step has been altered. Edit custom-made data views to select valid data sets
-  **384-** and **1536-well plates** require resizing to effectively see the data. Gen5 adds a button to the **Plate View** to zoom in on the top-left quadrant of the plate and zoom out to view the entire plate. After zooming in, use the scroll bars to bring the other quadrants into focus. Find more on resizing the views below.



- [Multi-index readings](#) offer another viewing option. Kinetic and scanning reads generate views based on the number of read intervals, wavelengths, or positions defined. Use the **spin** buttons or enter the desired read index and click **Show** to display it. Gen5 displays the time, wavelength, or position of the selected read number.

-  **Kinetic and Scanning protocols** can generate **Well Analysis** data sets labeled **Curves**, in the Matrix tab, open the Curves data set and click on a well for a [Well Zoom](#). (Note: 384- and 1536-well plates show a magnifying glass in the well instead of a curve.)

 Starting at the Curves data set, you can display multiple well zooms simultaneously by holding down the **Ctrl** key while selecting (up to 8) wells

- You can also select **Create new Matrix** to define a new view
- Select the **Statistics** tab to view a table of data reduction results
- Select the **Graphs** tab (when available) to view any [Curves](#), except kinetic analysis curves, which Gen5 calls Well Analysis and is described above
- Select the **Cutoffs** tab (when available) to view the values or results of the cutoff formulas
- Select the **Validation** tab (when available) to view the values and results of the validation formulas

Important Notes:

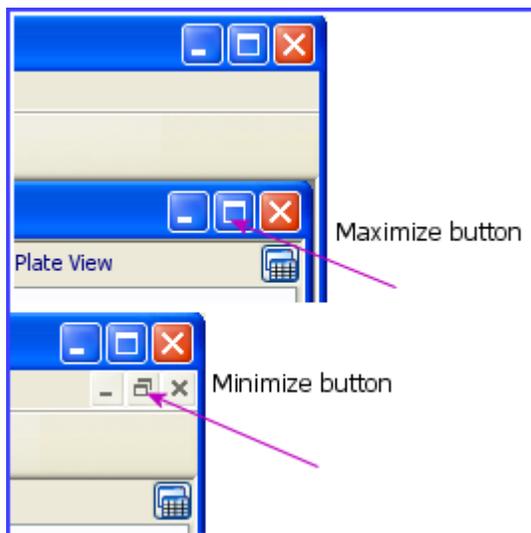
- Gen5 may not display some data points by default; to see them you must create your own [Data Views](#). If you expected to see certain results that are not currently displayed, try creating your own views.
- All data views are also available for Reporting and/or Exporting
- Modify a data view to change the way results are reported, including the number of decimal places and significant digits (page 313)
- Gen5 always uses your computer's **Regional Settings** to display and input data
- Learn the meaning of the [Symbols and Notations](#) displayed on page 307.

Opening the Plate View/Workspace

In an [Experiment](#), if it is not already open in the main view of Gen5™:

- from the menu tree: Double-click the desired  item
- Or, select **Plate > View**

Resizing the Plate View



Use the standard Windows® Maximize and Minimize buttons to resize the Plate View



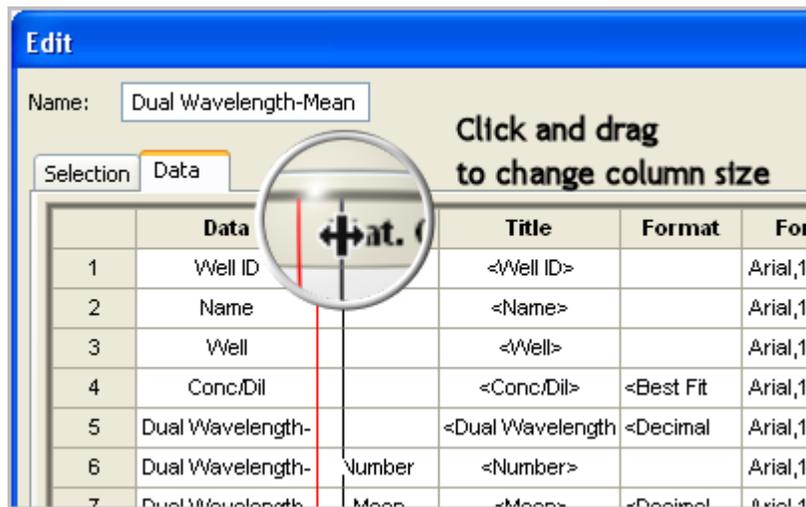
For more precise control, hover your mouse at the corner of the window, and with the two-headed arrow click and drag it to the desired size



You can also resize the columns and rows: hover your mouse on a border and drag the two-arrowed pointer to the desired column/row size

- ❖ Changing the window, column & row size typically enables the standard Windows® scroll bars, which you'll need to use to see all of the wells

Resize columns



 Hover your cursor over the division between columns until the cursor turns into a two-arrow I-beam. Then click and drag the column to the desired size. Just like Excel® and other programs! In some Gen5 views this option works for rows, as well.

Plate View (Workspace)

Plate > View

Use the **Plate View** or Plate Workspace to instantly view the results of an experiment and, if needed, to mask or alter the results.

About the Plate View

Gen5 always provides the Matrix and Statistics tabs, and when applicable, adds a Graphs, Cutoff Values and Validation tab

What you can do:



-  After reading the plate (or otherwise [acquiring data](#)), in the **Plate View** use the drop-down list for **Data** to select the data set (raw data or data reduction results) to display
-  Click the 3-dot button next to a data set to customize the view's appearance. This feature is also available in the [Data Views](#) dialog.
-  Click the **Quick Export** button to instantly open the current view in Excel®. Learn more about Gen5's [Export Options](#), including the right-click options: **Copy to Clipboard** and **Save As**

-  In the Matrix and Curve tabs, use the **Edit and Mask** buttons to change or mask selected data
-  In the upper-right corner, below the minimize, maximize, and close buttons, click the duplicate view button to open another instance of the Plate View. Use this feature to view the raw data results of a reading in one window and simultaneously display a curve plotted from the results in another window, for example
-  Use the read index **spin** buttons to consecutively advance the view of a Kinetic or Spectrum read, or enter a read/wavelength index in the field and click the **Show** button

How to set the default view

To determine the first data set or data view to open for each tab of the Plate View:

1.  Select **Data Views** from the menu tree
2. For each category, Matrix, Table, Graph, ..., **highlight the data** set you want to be shown first, i.e. during and immediately after reading the plate, when its corresponding tab is selected in the Plate View
3. Click **Set as default**

The **Set as default** button only appears when an eligible data set is highlighted. The button appears grayed-out when the current default is selected, and it disappears when a non-eligible data set or another item in the **Data Views** is selected.

Data Views

Protocol > Data Views

Gen5™ offers this menu-tree control box for selecting and modifying the way data is presented on-screen and in reports and export files. The selections and modifications made for on-screen viewing (Data Views) become the settings for the [Report Builder](#), [Power Export Builder](#), and [File Export Builder](#)

 **Create your own Data Views** for the most precise control of content and appearance.

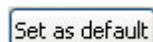
Gen5 lists all the data sets available for viewing under a display category:

- **Matrix** = a grid that represents the microplate
- **Table** = a columnar presentation of the data
- **Graph** = a standard curve or well zoom generated from a Curve or Well Analysis
- **Field Groups** = user-selected or defined data points to include in reports



The function buttons: **New**, **Edit**, and [Hide/Show](#) are enabled or disabled in sync with your selections in the menu tree.

- **Highlight** an item in the tree and click **Edit** to modify its appearance. Depending on the type of item, you can change its:
 - **Numeric Format** and **Font**, e.g. number of decimal places,
 - **Range of data**/wells to include in views and reports,
 - **Sort order**, layout and other characteristics.
- **Highlight** a display category to enable the **New** button. Modifying the system-provided views is limited, but you can create your own views to display exactly the data elements of interest, in the most pleasing format.



The **Set as default** button only appears when an eligible data set is highlighted. The button appears grayed-out when the current default is selected, and it disappears when a non-eligible data set or another item in the **Data Views** is selected. Use the button to determine the first data set to open in the Matrix tab of the Plate View. Gen5 displays the first raw data set (measurements obtained from the reader), unless another data set has been set as the default.

Data Set Naming Convention

Gen5™ names the data sets it creates this way:

Name	Meaning	Example
wavelength	Absorbance wavelength	450
wavelength[#]	When 2 or more identical read steps are defined in the same Procedure, the wavelengths are numbered	450[2]
ex/bw & em/bw	Fluorescence filter sets: excitation/bandwidth and emission/bandwidth	360/40, 580/40
em/bw	Luminescence emission wavelength/bandwidth	460/40
Lum	Luminescence using Hole/empty position in the filter wheel	Read 3: Lum
Spectrum	Spectrum scan	Read 1: Spectrum
Curves [nm] Curves [ex/bw, em/bw]	Kinetic curves are automatically generated during kinetic analysis; they lead to Well Zooms	Curves [490] Curves [360/40, 460/40]
Read #: nm	When more than one read step is defined, the Read number names the data set	Read 2: 410
Blank nm	Blank well subtraction data set (created automatically by Gen5)	Blank Read 2: 360/40
Corrected [nm]	Pathlength corrected data set (Absorbance read option)	Corrected [405]
Polarization	Data transformed per fluorescence polarization formula	Polarization
Parallel Intensity	Raw/ blanked data from parallel measurement	Parallel Intensity
Perpendicular Intensity	Raw/ blanked data from perpendicular measurement x G Factor	Perpendicular Intensity

ex/bw,em/bw [Parallel] or [Perpendicular]	Raw data from parallel or perpendicular measurement	485/20, 590/35 [Parallel] 485/20, 590/35 [Perpendicular]
Anisotropy	Data transformed per anisotropy formula	Anisotropy
<p>Data Reduction steps produce data sets named with the type of calculation and the raw data set name. For example, Well Analysis steps could generate: Mean V [340/40, 520/40] and Onset [Blank Read 2: 490]</p>		
<p>When a Step Label is entered for the Read or Data Reduction Step, the label is included in the name of the data set. For example: a Step Label in an Absorbance read step of Test1 would result in a raw data set of "Test1:450" and an automatically-generated blank-subtraction data set would be "Blank Test1:450".</p>		

Symbols and Special Notations

Depending on the current view, report, or exported text you may see any of the various symbols and notations that Gen5 applies to call your attention to special conditions:

Symbol	Description
>(highest conc.) <(lowest conc.)	After fitting a standard curve and calculating the concentrations, Gen5 denotes wells falling outside the abscissa range with >(upper limit concentration) or <(lower limit concentration); <0.00 is displayed rather than negative concentrations, unless it's called in an interpolation♦
?????	????? appears in a well in certain Matrix and Statistics views when data for that well has not been obtained; cannot be calculated; is based on out-of-range values; or has been failed by the reader
####	#### appears in a well when the size of the grid is not large enough to show the value in the well. Resize the view to see the contents. Or, change the Font used to display the value, choosing a different "clipping" method
[]	In Well Zoom views when the original read range is reduced, Gen5 plots the revised Calculation Zone with brackets: []
1234	Gen5 displays Masked values in data views and reports between asterisks
!1234!	When Gen5 uses an out-of-range value in statistics calculations, it is considered a biased result and will be reported between exclamation points♦

*	Asterisks are displayed next to plate icons and in the title bar of an experiment when a change is made or an action is taken but the file has not yet been saved
** dataset**	Asterisks identify invalid data sets. This is generally caused by changing the source Read step or Data Reduction step after the data set was initially created
#N/A	Indicates an invalid data reduction item
INJECT	Synchronized Mode Procedures with multiple, appended kinetic loops and one or more dispense steps, use this symbol to identify when dispensing occurred
OVRFLW	Measurements are not being collected, most likely due to inappropriate read parameters for the test specimen (overflow)
MISSED	The reader did not capture a value for every Xenon flash measurement taken for this well. The reader missed a measurement and cannot report a reliable value.

❖ **Note:** Biased values may appear in a Statistics table when **Calculation Options** are set to include out-of-range values. If, for example, the concentration for one of three standard replicates is reported as >40, the Standard Deviation and CV% values are considered suspect. Gen5 surrounds the values with exclamation points to indicate that it is the user's responsibility to determine whether or not the results are valid.

Well Analysis Results Table

When a Well Analysis Data Reduction step is defined, Gen5 shows the calculation results in a table beneath the curve. The "Curves" or "Scan" data set that leads to a well zoom, must be the subject of a Well Analysis step; raw data well zoom views do not include a Well Analysis Results table.

Calculation Zone

The range of reads considered for analysis is determined by the Calculation Zone setting of the Well Analysis. When the original range is reduced, Gen5 plots the revised Calculation Zone with brackets: []

Viewing Appended Kinetic Results

When one or more kinetic reads are appended, Gen5 combines them into one data set. When a Dispense step occurs between the kinetic loops, Gen5 represents the event as a blue diamond on the X-Axis timeline.

❖ **FLx800 with Injectors** may show the "Dispensing" event occurring in the same interval as a Read. This is a limitation of the reader's basecode or on-board software.

Data Points Reference

When customizing or creating a new view in Gen5, the following data points may be available depending on the Procedure (reading parameters) and Data Reduction steps:

Data	Description
Well ID,	Plate Layout well assignments
Conc/Dil, Conc/Dil type	Concentration or dilution values and setting in Plate Layout
Unit	User-defined in Plate Layout
Name	Sample IDs
Bitmap [<i>nnn</i>]	Kinetic/Spectrum Curves or Area/Linear Scan image
Formula [<i>nnn</i>]	Transformation formula
Label: <i>nnn</i> [<i>nnn</i>]	User-defined labels for read and data reduction steps

Gen5's Tables

About Gen5's Tables

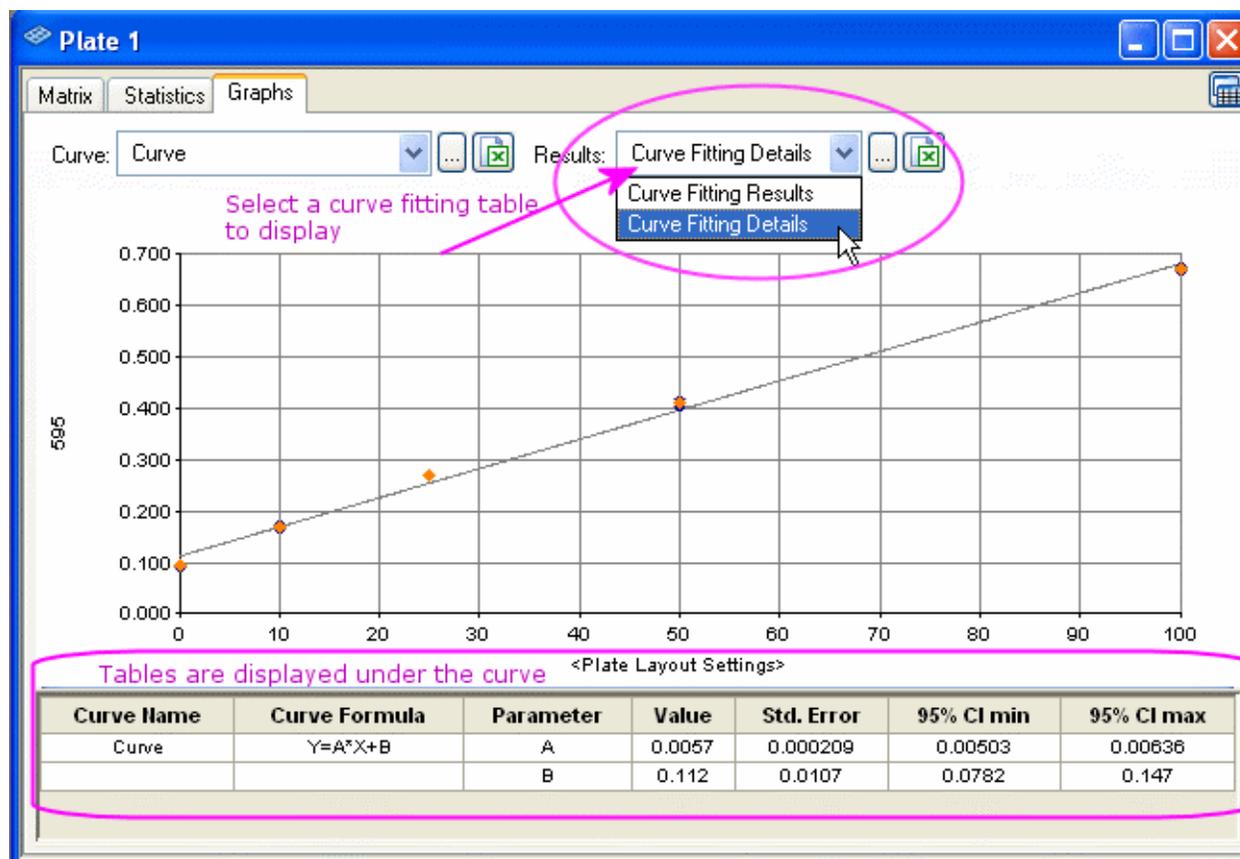
Based on the type of plate reading and the data reduction steps defined, Gen5 automatically creates tables to display, report, and export the data. You can make slight modifications to the system-generated tables, like changing the font and numeric format of an item. And, you can create your own -custom-made- tables.

You can view the tables in the [Plate View](#):

- Statistics tables can be viewed using the **Statistics** tab,
- Curve Fitting results are shown below the curve in the **Graphs** tab,
- Well Data and Well Results tables can be viewed in the [Well Zoom](#).

Some tables, like Validation results, can only be printed (or **Print Previewed**) and exported.

Curve Results Table



Potential Tables

Depending on the protocol, specifically the type of read and data reduction steps, and the level of Gen5 software, the following tables may be available for viewing, reporting and exporting data.

- **Statistics** — show the results of a data reduction step. The system-generated tables display the **Well** (well coordinate), **Well ID**, and **Concentration/Dilution** assigned in the Plate Layout; the Sample IDs or **Name** assigned to the Well ID; the **Count** or number of replicates; and the **Mean**, **Standard Deviation** and **Coefficient of Variation** percentage (%), if available.
- **Curve Results** — Gen5 generates three tables to show the curve fitting [Parameters and Values](#) of a curve. The "Curve Fitting Results" table includes the curve name, formula, parameters, and R². The "Curve Interpolations" table shows the interpolation data. And, the "Curve Fitting Details" table lists the parameters and their calculated value, standard error and 95% confidence interval. You can display these tables underneath a curve in the Graphs tab of the Plate workspace when viewing them online. You should review the details to assess the goodness-of-fit of your curve. Depending on the Curve Fit Method, numerous other parameters and values can be displayed/reported in a custom-made table.
- **Well Data** — show the well-specific details for multiple-reading Read Steps like kinetic analysis, spectrum, and linear scans. **Well Data 2D** is the Area Scan rendition. They are used in [Well Zooms](#) to toggle the display between View Data and View Chart. When adding this kind of table to a report or export file, it is necessary to select the specific wells, by their well coordinate, e.g. A1, that you want reported.
- **Well Analysis Results** — show the generated data of a Well Analysis Data Reduction step. They are viewed in a related [Well Zoom](#), beneath the curve. Note: The "Curves" or "Scan" data set that leads to a well zoom, must be the subject of a Well Analysis step; raw data well zoom views do not include a Well Analysis Results table. When adding this kind of table to a report or export file, it is necessary to select the specific wells, by their well coordinate, e.g. B2, that you want reported.
- **Cutoff Results** — show the cutoff formula as defined and the value of the formula. While the results of the cutoff criteria can be viewed online, using the **Symbols** data set, the Cutoff Results table can only be reported and exported. You can use the Print Preview feature to view this report component online.
- **Validation Results** — show the validation Formula as defined and the Value result of the formula. The Validation Results table can only be reported and exported. You can use the Print Preview feature to view this report component online.
- **Audit Trail** — shows the entries, i.e. change history, logged in the Audit Trail. Depending on your level of Gen5, one or more types of audit trails are maintained and can be reported: Data, Protocol, and Calculation Warning. This

table is only used for reporting and exporting, as Gen5 offers the [Audit Trail](#) viewer in the menu tree for online viewing. The audit trails tables list date, user, event and user's comment logged at the time of the event.

- **Signatures** — shows the date, time, reason and signatory of each signing event. Also included is a "Document modified" record whenever the protocol or experiment is changed after a signing event. Refer to the audit trail to determine how the file was changed.
- **Procedure Summary** — lists the steps, in sequence, defined in the StepWise Procedure for reports and export files

 When building reports, using the [Report Builder](#), group together related items, like the Curve and its Curve Results table, and a Well Zoom and its Well Data, for the best results.

Print Preview to see Tables

Cutoffs (Blank A1[450]-R-Squared)			
Formula		Value	
.85		0.85	
1		1	

Validation (A1(450))			
Formula		Value	
STD1>0.200		Valid	
0.900<STD2<1.300		Invalid	
1.3<STD4<2.5		Invalid	

Data Audit Trail			
Date	User	Event	Comment
02/07/05 10:28:36	Administrator	Plate read started	
02/07/05 10:28:56	Administrator	Plate read successfully co...	

Calculation Warnings
Error/Warning messages
Validation (A1(450)): At least one validation condition failed.

This is a sample report of the Cutoffs, Validation, and Audit Trail tables, using Print Preview.

Modify/Customize Views/Data

Numeric Format for Results

Gen5 provides controls for changing the numeric format for results. You can choose between **Scientific notation**, **Decimal** and **Best Fit** formats. Then you can define the number of significant digits or decimal places to display/report. Scientific notation is also known as Standard index notation.

How to change the numeric format:

The numeric format is set individually for each applicable data set.



After defining the protocol, open **Data Views** (select **Protocol > Data Views**):

1. **Highlight** the data set you want to format in the tree and click **Edit**
2. Select the **Data** tab
3. In the **Format** column, click inside the applicable table cell (row) to enable a **3-dot** button that leads to the **Numeric Format** control for that data point
4. Click the **3-dot** button and use the controls to define the format. Select a **Format** and define the level of **Precision**. The **Samples** section of the control shows the affect of your selections on sample data.

❖ Data views/data sets can also be **Edited** in the Plate View and after they have been added to report and export content. Click the 3-dot button next to the Data field of the Plate View. Double click an item added to the Report/Export Content in the respective builder.

Gen5's Numeric Formats explained:

Gen5 provides these display options for numeric results in reports and on-screen. "Display options" being the operative description. When performing data reduction operations, Gen5 uses all the digits (up to 15) regardless of the numeric format applied for display. Use the **Round(x;y)** and **Truncate(x;y)** functions to control the number of digits used in/generated by a calculation.

Format options:	
Decimal	Standard, unmodified numeral
Scientific Notation	A way to write very small or large numbers. Numbers are separated into two parts, a real number with an absolute value between 1 and 10 and an order of magnitude value written as a power of 10. The '10' is omitted and replaced by the letter E or e, which is short for exponent.
Best Fit	Gen5 determines, based on the size of the display area, the format, Decimal or Scientific

After selecting the format, define the level of precision for the display of numeric results. Select and set the number of **Decimal places** or **Significant digits**:

Example	Decimal places = 3	Significant digits = 3
0.000123456	0.000	0.000123
12.34567	12.345	12.4
123456	123456.000	123456

Modify Data Files

After data has been retrieved from the reader (or entered manually, or imported from a file), Gen5™ permits authorized users to **edit** or **mask** measurement values. Gen5 automatically logs an event in the experiment's Data Audit Trail any time data is changed or masked.

❖ **Important!** Changing or masking wells may significantly alter or even invalidate results.

- Only raw measurement values can be changed. Data sets generated from Data Reduction calculations cannot be changed, only masked.
- When a well is masked, Gen5 excludes its value from further calculations.
- In most views and reports, masked values appear sandwiched between asterisks, e.g. *6879* and edited values are shown in parenthesis, e.g. (6879)

Change a Measurement Value

After data has been retrieved from the reader, Gen5™ permits authorized users to **edit** or **mask** measurement values. Gen5 automatically logs an event to the file's Data Audit Trail any time values are changed.

1. With the experiment open, and the desired plate selected in a multi-plate experiment, open the Plate workspace
2. In the Plate workspace, select the **Matrix** view and from the **Data** drop-down list, select the desired data set (it must contain raw measurement values); or in **Well Zoom** view, click the **View Data** button
3.  Click the **Edit** button

❖ The System Administrator can protect the use of this function. If the button is grayed out, you may not be authorized to use it.

4. Click on and change the value of one well at time in the grid. Multiple wells can be changed in a session.

5.  To apply the changes, click the **OK** (green check mark) button. Gen5 will recalculate results.
-  Click the cancel button to ignore your entries, and restore the original values.

Mask (Exclude) a Data Point

Most data views let you mask or ignore one or more data points:

1. With the experiment open, and the desired plate selected, open the Plate workspace
2. In the **Plate** workspace, select the desired view, Matrix or Graph. Depending on the view, click the **Data** or **Curve** drop-down list to select a data set. Or go to the [Well Zoom](#) view, where you can either **View Data** or **View Chart**
3.  In the Matrix/View Data view, click the **Mask** button. (This is not necessary in [Curve/Chart](#) view.)

❖ The System Administrator can restrict the use of the Mask function. If the options are grayed out, you may not be authorized to use it.

4. Click a well in the matrix or a point in a curve to mask it. (Click again to unmask.) Multiple data points can be masked in a session.
5.  To apply the changes, click the **Apply** (green check mark) button to recalculate results.
-  Click the **Cancel** button to ignore your entries, and restore the original values.

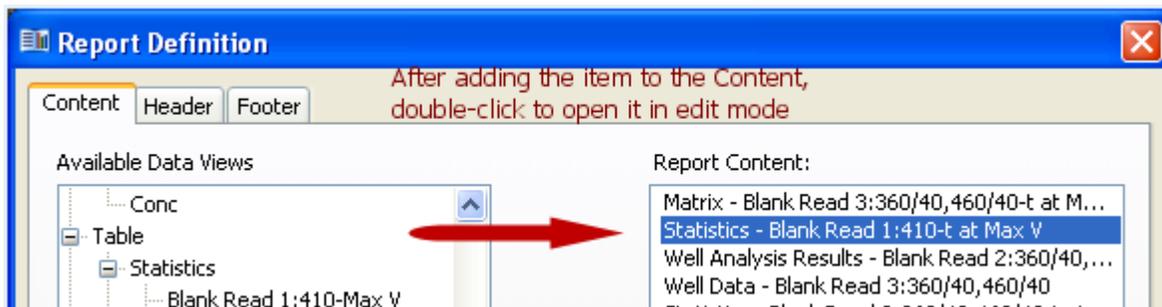
 In curve views, **right click** or **click & drag** for more masking options: you can mask and unmask multiple points simultaneously

❖  **Mask** and **Edit** functions are done in a "session." If you change the view, e.g. change the Read Index in a kinetic analysis, or close the plate view, experiment, or Gen5, without first applying the changes, Gen5 prompts you to **Apply** or **Cancel** the modifications you've made, before it can close the session.

Change the Time Format

For viewing and reporting Kinetic or Well analysis results, you can change the time format using Gen5's Edit tool for data views.

In the Report and Export Builders:



(Not shown: In Data Views, highlight the item and click **Edit**.)

On the Data tab, in the Format column for the data point,

	Data	Stat. Op.	Title	Format
4	Conc/Dil		<Conc/Dil>	<Best Fit,5>
5	Blank Read 3:360/40,460/		<Blank Read 3:360/40,460>	<HH:MM:SS>
6	Blank Read 3:360/40,460/	Count	<Count>	
7	Blank Read 3:360/40,460/	Mean	<Mean>	<HH:MM:SS>...
8	Blank Read 3:360/40,460/	Std Dev	<Std Dev>	<HH:MM:SS>
9	Blank Read 3:360/40,460/	CV (%)	<CV (%)>	<HH:MM:SS>

After defining a **Procedure** and **Data Reduction** steps based on a time format:

1. Open the **Data View**, **Report Builder**, or **Export** file builder, and locate the Matrix, Table or Curve that contains the data you want to modify and click **Edit** or **double-click to open the item**. (In Report & Export Builders, first **Add** the view to the selected Content box.)
2. In the Edit dialog:
 - for a **Matrix** or **Table** select the **Data** tab and locate the time format you want to change in the **Format** column, then, click inside the cell to enable a 3-dot button
 - for a **Curve** select the **X-Axis** tab, locate the Format field under **Labels**
3.  Click the 3-dot button in the **Format** column/field to open the **Time Span Format** selector.
4. Fill the checkboxes to select the desired time-format options.

Modify protocol parameters in an experiment:

After a plate is read, the Procedure cannot be changed. Other changes to the experiment are permitted:

1. With the experiment open, select an option from the Protocol menu tree, other than Procedure. Double-click the element you want to modify, such as Plate Layout, Data Reduction, Report, and so on.

❖ **Warning:** Making changes to the Protocol elements may have an unintended effect that ripples through your protocol. For example, deleting or altering a Data Reduction step whose results are used in a subsequent step, could invalidate the subsequent step.

2. Within the appropriate protocol dialog, make the desired change and click **OK** to recalculate results.

❖ The System Administrator can protect some or all protocol parameters from being modified. If you are trying without success to modify these parameters, you may not be authorized to do so.

Gen5 Secure automatically updates the Audit Trail to reflect any changes made.

Modify the Matrix View

You can change the number of decimal places or significant digits expressed and customize the font used, among other options.

There are two ways to access this feature:

1

Select **Protocol > Data Views**, highlight the desired data set under Matrix in the menu tree and click **Edit**

2

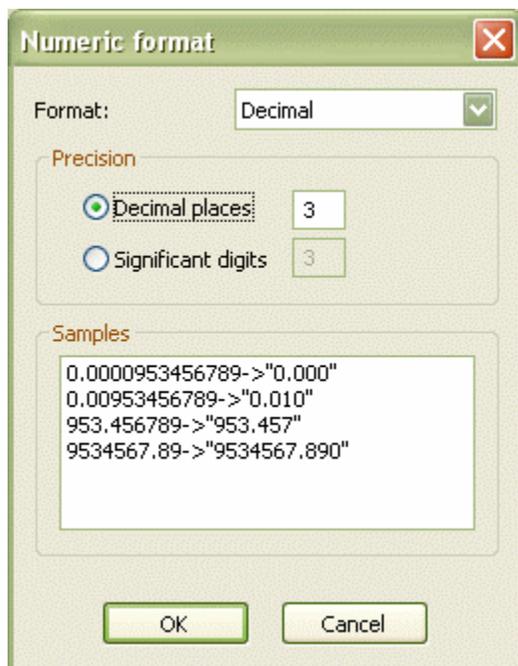
 In the **Matrix** tab of the **Plate View**, select the **Data** to modify and click the 3-dot button

 **Report** and **Export** Builders offer the same options. Generally, it is more efficient to modify the matrix using these approaches, so you see the changes both on-screen and in the Report and Export outputs.

Edit

Except for custom-made matrix views (Create New Matrix), modification options are limited to Format, Font, and Selection

Format



Click inside a cell in the **Format** column to enable a **3-dot** button, and click on the **3-dot** button to open the Numeric Format dialog.

You can select the desired **Format** using the drop-down list and set the number of **Significant digits** or **Decimal places**

Use the **Samples** window to judge the effect of your selections on the appearance of the data

Font

To change the font used to display and report values: click inside a cell in the **Font** column to enable a 3-dot button that leads to the Font dialog. Use this standard Windows® Font option to make selections.

Title

Customize the Title of the data set by clicking in the field and typing over the Auto or default text. The title appears as a [Tool Tip](#) when the mouse is hovered on a data point in the Matrix view.

Selection

Modifying a matrix via **Data Views** and the **Report/Export Builders** offers the Selections tab. It is most useful in multiple-read experiments, e.g. kinetic, that produce multiple results sets. Use the Selections tab to define a range of results sets to include in reports and export files.

Modify a Graph

Gen5™ provides tools to change the appearance of a graph. You can display or hide the legend and change the title, labels, font, and colors.

There are two ways to access this feature:

1

Select **Protocol > Data Views**, highlight the desired curve (under Graph in the menu tree) and click **Edit**

2



In the **Graphs** tab of the **Plate view**, select the curve to modify and click the 3-dot button

The **Report Builder** offers the same options. Generally, it is more efficient to modify the graph using these approaches, so you see the changes both on-screen and in print.

Layout

Select the Layout tab to:

- Define a **Title** for the curve; position the title on the page/screen; and select the font
- Display or hide the **Legend**; and select the font for the legend

X Axis/Y Axis

On the X Axis and Y Axis tabs, you can:

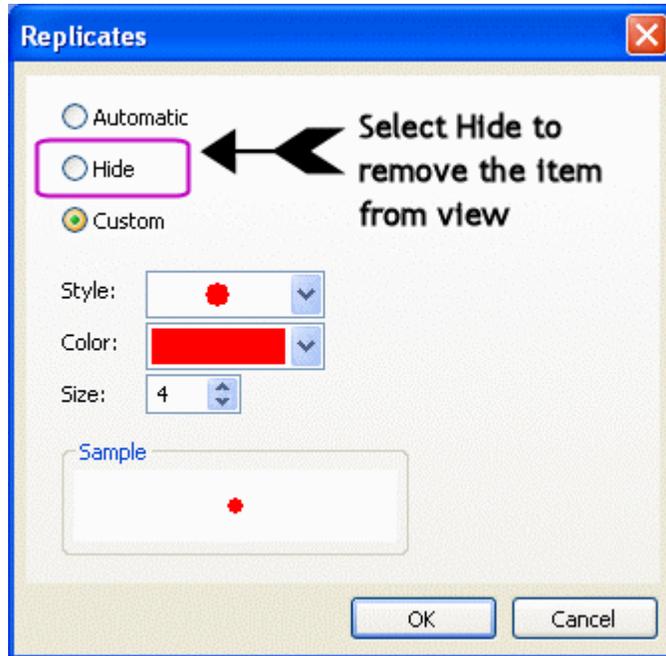
- Define a **Title** or label for the axis; select the font; **Show** (or hide) **Gridlines** for the axis;
- Change the **Scale** of the display using **Linear** (1, 2, 3) or **Log** (10, 20, 30) numbers; **Auto** applies the method selected when the curve was created in the [Curve Analysis](#) step of Data Reduction
- Change the numeric **Format** and **Font** of the **Labels**, i.e. the numbers/data displayed on each axis. Try out the various options to determine which combination works best for your reporting and viewing requirements.

Click the 3-dot button for **Format** to change the scientific notation, decimal places or significant digits displayed in the view/report/export

Plots/Curves

Select the **Plots** tab to further modify the appearance of the graph. You customize the appearance of the error bars and fitting line, for example. (The **Curves** tab is displayed for user-created curves, select a curve and click **Edit** after to enable these functions):

-  Click in a cell in the table to enable a 3-dot button that leads to formatting options for the selected item (line, marker, text, or pattern):
 - Automatic** is the default setting. It is most useful in Well Zooms, when more than one well is selected for simultaneous viewing. Gen5 displays a different color scheme for each well selected.
 - Select **Hide** (or None) to remove the item from the view.
 - Select **Custom** to modify it. Use the drop-down options to apply different colors, symbols, styles and/or line weight.



 Select the **Plot** tab to turn-off the **Text** that may be obscuring the details in a **Well Zoom** curve: select the table cell containing the offending text, click the activated 3-dot button. At the Text tab, select **Hide**

Chapter 15

Reporting Results

This chapter describes Gen5's **Report Builder** for generating print-ready data output for your experiments. Instructions for creating **Field Groups** to report inherent data points, how to change the Font, and other useful tips are provided.

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Building Reports

Protocol > Report Builder

About the Report Builder

Gen5™ provides this tool to define exactly:

- what to include in the report
- how to format an item in the report
- where to place the item in the report.

 Define the **Data Views** and **Default Protocol** to speed up this step! Create **Headers** and **Footers** and add the **Protocol Summary** report sections in the Default Protocol so they appear in all newly-created protocols.

Report elements should be defined for each Protocol:

1. In the menu tree, under **Protocol**, select **Report Builder**
2. Highlight **Available Data Views** and **Add** them to the **Report Content** box
 - You can also **Drag and Drop** items into the Report Content box
 - Drag and drop to change the sequence order of items in the Report Content box
 - **Edit** an item in the Report Content box with a double click or use the right-click menu
 - Highlight an item in the Report Content box and click **Remove** to de-select it (or use the Delete key)
 - To clear the slate and start over, click **Remove All** to de-select all items.
 - **In multiple-read (multi-index read) protocols**, like kinetic analysis, it is necessary to select a range of reads or a range of wells to include in an export item. Certain data elements, like Well Data, Well Results, and Well Zoom, **require manual selection** of the specific wells to include. Except for area scan results, you can select multiple wells for simultaneous reporting in the resulting table or graph. For details see [Reporting Well Analysis Results](#) on page 330.

 Gen5 opens report items in **Edit** mode when a selection is required. For example, when you select a Matrix item to report a multi-index/kinetic read, you must select a Range of read numbers. One matrix or grid will be reported for each read number.

3. Highlight an item in the Report Content box and click **Page Break** to add a page break to the report. Gen5 inserts it just above the highlighted item. You can drag and drop the Page Break to move its sequence order. (This step often works best after you've read the plate and can use **Print Preview** to assess the layout of the report.)
4. Set up [Headers and Footers](#), if desired. (page 339)
5.  When the report is defined, in an experiment, click the Print button to generate the report.

- ❖ ** Items marked with asterisks indicate 1 of 3 conditions:
 1. You must [select](#) specific data points from a very large (multi-read) data set or
 2. The experiment did not generate the expected results, an error occurred or
 3. The Procedure and/or Data Reduction steps that generated the item have been changed making it invalid **

 Click **Print Preview** to assess the report layout!

Using the Default Protocol

Using the controls in the Default Protocol, you can input text and select protocol and experiment data to be included in all your reports. Your selections can be modified, removed, or replaced in individual protocols without affecting other experiments.

System > Preferences > Default Protocol > Report Builder

- ❖ Content options in the Default Protocol may be limited by the lack of a defined Procedure.
- ❖ **Important: Default Protocol Settings** are applied "going forward," they do not affect existing protocols. They are applied only to newly-created protocols.

How to create and customize a report

These step-by-step instructions are intended to introduce you to Gen5's tools and method for outputting results. While this example uses the Report Builder, the options and process is the same when building export files.

Important first step

You must define the [Procedure](#) (read parameters) and the Plate Layout before you can define Data Reduction steps. Likewise, you must define these and other elements of the [Protocol](#) (to generate the content of reports and export files) before you can create and customize the report.

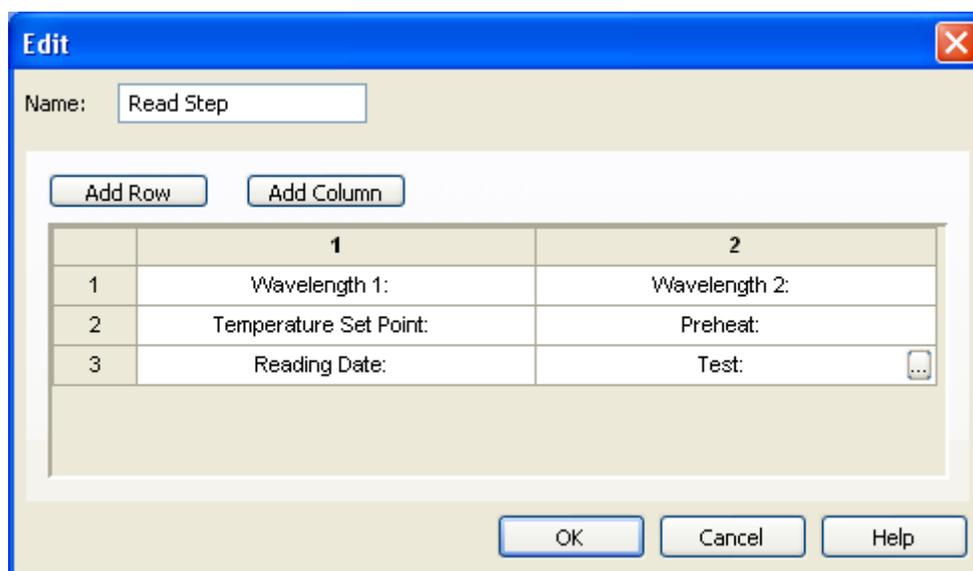
After you've defined the Procedure, Plate Layout, Data Reduction, and Runtime Prompts, you're ready to build the report.

Create a Field Group to report details

Field Groups can be used to report all of the details affecting your experiment:

1.  Open **Data Views** from the menu tree
2. Highlight the **Field Group** label at the bottom of the tree and click **New**
3. Enter a **Name** for the field group at the top of the Edit dialog. For this exercise, we'll name it Read Step.
4. Click in the first cell of the table to enable a 3-dot button, and click the **3-dot** button. The **Fields** dialog opens.
5.  Use the drop-down list to set the **Category** to Procedure for this exercise.
6. At **Field**: use the drop-down list to select a data point. Select Measurement 1 for this exercise.
7. At **Label**: click in the text field, and overwrite the default label with a more fitting label for this data point. For this exercise, we'll call it Wavelength 1 because we have multiple wavelengths.
8. Click **OK** to close the Field dialog.
9. Repeat steps 4-8 for the other cells in the table, adding Rows and Columns as needed. Explore the fields offered in the other Categories, like Plate Information, to find all the details you want to include. When you've added all the desired fields, click OK to save and close the Field Group.
10. At **Data Views**, click **Close**.

Here's an example of the Field Group:



Build the Report

1.  Open the **Report Builder** from the menu tree
2. Locate the **Field Group** category at the bottom of the tree and highlight the just-made field group (Read Step for this exercise). User-defined field groups appear in bold text. Click **Add** to include it in the **Report Content**.
3. Scroll up the tree of **Available Data Views**, selecting and adding the desired items to the **Report Content**. The order of the items listed in Report Content is their order in the printed report. [Drag and drop](#) items to change their position.
4. After adding an item to the **Report Content**, double-click it to open it in **Edit** mode. Now you can customize the output. For example, on the Data tab, in the **Format** column, click in the cell to enable a 3-dot button that lets you change the number of decimal places or time format; on **Selection** or **Well** tabs define the range of data to report. Editing **Curves** lets you define the X- and Y-Axis titles. You can even combine multiple curves in one graph, if applicable.

 In Edit mode, change the **Name** at the top of the screen to customize the section heading in the report

5. Click **OK** to save your work and close the Report Builder.
6.  If you're performing these steps in an experiment, click **Print Preview** to assess the report layout. If you're performing these steps in a protocol, save the protocol and create a new experiment based on it. Then use Print Preview to judge the output. Return to Report Builder to make any changes to the selected content.

Customizing Reports

Gen5™ offers extraordinary flexibility in customizing reports and export files. Almost everything you can view in Gen5 can also be reported. And, once you learn a few basic steps, you'll be able to define exactly what to include and exclude from reports and on-screen views.

Basic Rules:

- **Data elements** that are created or modified in **Data Views** become available for selection when defining the Report and Export parameters. This includes hiding a data set, which removes it from selection. Setting up the views in the **Default Protocol** (if possible) makes them available in all newly-created protocols.
- **Create your own Data View** for the most precise control over content and appearance. You can create a matrix, table or graph that displays and reports the information you've selected. You can include multiple data sets in one view, for example. And, exclude default data points that do not apply to your experiment.
- **Include the Protocol Summary options:** Procedure and Data Reduction Summary to report protocol elements used to obtain the measurements being reported. They are a good candidate for the Default Protocol.
- **Create Field Groups to include miscellaneous information and data points** in your reports/export files. Gen5 captures numerous details like temperature and shake duration, Plate ID and other run-time entries, and print date and page count. Virtually all of the details affecting your experiment can be reported.
- **In multiple-read protocols**, like kinetic analysis, it is necessary to select a range of reads, or a range of wells to include in a report item. Certain data elements, like Well Data, Well Analysis Results, and Well Zoom, **require manual selection** of the specific wells to include in the report. You can select multiple wells for simultaneous reporting in a table or graph.
- **Select the Font option** when editing content to define the "clipping" method to apply to data points that are too long to fit completely in a report column. Several choices are available to hide, truncate, or display as much as possible of an extra-long data point.
- **Select the Format option** when editing content to define the number of decimal places or significant digits to report or to change the time format of a report item.
- **Attributes applied to data elements** in Data Views and the Report and Export Builders take effect going forward. They do not replace, update, or overwrite an item that has been previously assigned to a Report/Export. You must **refresh** the selected contents after making any changes to a data

element. And, any previously-saved Experiment will not reflect the content or formatting changes.

- **When you Edit** data elements in the Report Builder, your changes only apply to the Report. They will not be reflected in the Data Views or Export options. And, unless you use the **File>Save Protocol As** option, your changes will not be applied to any future experiments based on this protocol.
- **384- and 1536-well plate matrixes** are reported in segments that best fit the data to the page. Generally, the first 12 columns are reported in one matrix, the next 12 columns in another and so on, unless the page orientation is changed to landscape or the font size is reduced. Unless otherwise directed, Gen5 reports the entire matrix on as many pages as needed to display all the data.

Best Practices:

- **Create your own Data Views** for the most freedom-of-choice in reporting and online viewing.
- **Print Preview** your reports to make sure you're happy with the layout before printing them.
- **Curve Results** tables work best just below or above the curve they describe. Drag and drop the Curve or the Curve Results in the Report Content to arrange them this way.
- **Limit the number of wells** in a Well Zoom graph to 8 for best results.
- **Changing the page orientation** from "portrait" to "landscape" may be the best use of the space and improve the appearance and readability of the report, especially when reporting 384-well and larger plates.

To change the page orientation:

1. Select **File>Print Setup...**
2. Select the desired **Orientation**

❖ You must have the experiment file open to change the Print Setup

Reporting Well Analysis Results

Protocol > Report/Export Builder > for the selected Content

By its nature, the individual well results of Well Analysis make reporting them a bit tricky. Well Analysis results, like Well Zoom graphs and Well Data tables, require an extra step when defining reports and exports. See **Report Examples** on page 332.

In the Report Builder and for applicable export routines, in the Export Builders, when you select any of the following:

- Well Data table
- Well Data 2D table (for area scans)
- Well Analysis Results table
- Well Zoom graphs
- Area Scan Zoom graphs

▶ **Be sure to open (Edit) the item to select one or more wells as the content to report/export.**

In the Report or Export Builder:

1. Add a well analysis item from **Available Views** to Report/Export **Content**
2. If the item doesn't open in **Edit** mode, double click it
3. Select the **Wells** tab or the **Curves** tab (depending on the type of report item)
4. **Select one well at a time** to report on: enter a well location in the **Well** field; Gen5 displays the Well ID and concentration or dilution assigned to the location, or
Select multiple, adjacent wells using a hyphen to define the range, A1-H12, for example, would include all wells in a 96-well plate
Important: Some report items require limiting the number of wells that can be successfully included in an instance of it. You may need to add multiple instances of the item to report all the desired wells. Use **Print Preview** to determine how many wells can fit on the page.
5. Click **Add** to move the well into the **Selected** box. Except for **Area Scan** results, you can select multiple wells for each report item. (See [Tip for Area Scan](#) below.)

- ❖ Tables and Curves respond differently to this treatment. Curves: **Well Zoom** graphs, overlay the results for each well in one curve. (Limit the number of wells to 8 for the best results.) **Well Data** tables present the data for each well in a column or row, adding another column/row to the table for each well. **To best fit the data in a report it is often necessary to add multiple instances of an item.** And, for each instance select the maximum number of individual data points that fit cleanly. Likewise, to report well zoom graphs individually, rather than overlaying them, you must add multiple instances of the Well Zoom graph. Use **Print Preview** (after the plate is read) to determine the best fit for your data. It's a "trial and error" process, selecting and revising the Report Content, and assessing the layout with Print Preview, until the report is satisfactory.
- ❖ Exporting the data does not require the same level of precision when selecting content. Gen5's File Export Builder delimits data points without restriction (and does not support curves) and Power Export exploits Excel's spreadsheet capabilities to handle limitless data transfer.

6. (Optional when available) Highlight a well in the **Selected** box and click the **Edit** button to modify its formatting.

You can remove multiple wells from the Selected box simultaneously: hold the **Ctrl** key while selecting non-contiguous wells or hold the **Shift** key while selecting adjacent wells, then click **Remove**

Tip for Area Scans

You must add **multiple instances** of the Area Scan Well Zoom and Well Data 2D table to the **Content** to report /export multiple area scan wells. For best results, identify the selected well in the **Name** field when adding each well, one well at a time, to the report content. The Name becomes a sub-heading in the report output, so you can easily distinguish between reported wells, e.g. Scan: B3.

Report Examples

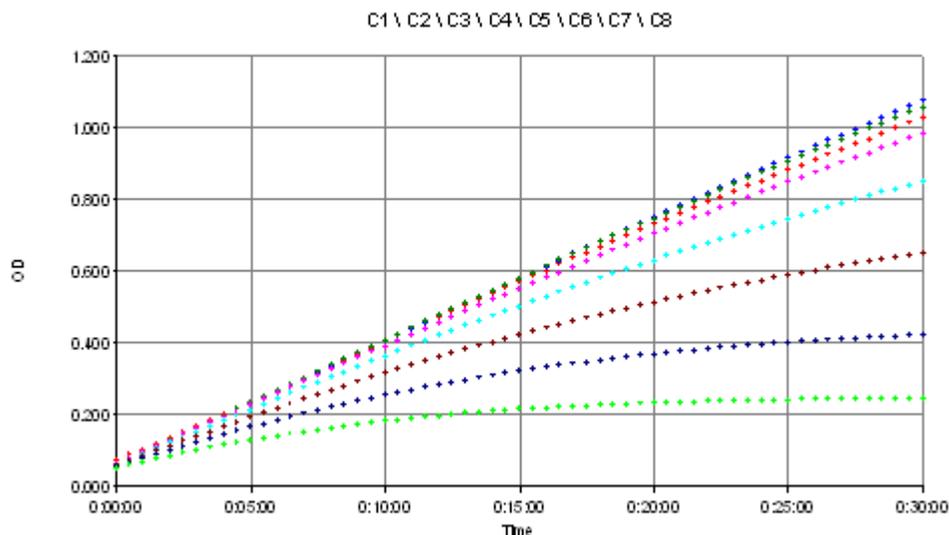
Here are some sample report outputs of well analysis results:

Using Gen5's Report Builder to output well analysis results involves some trial and error to determine the maximum number of wells that can fit neatly on the page. Here are some examples of the offerings:

Well Zoom Graph

420

This is a sample Well Zoom graph with 8 wells selected



Limit the number of wells to 8 when reporting a well zoom graph for the best results

Well Data Table

420

This is a sample Well Data table

Kinetic read	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
0:00:00 s	0.069	0.062	0.057	0.056	0.057	0.056	0.056	0.049	0.044	0.038	0.034	0.031
0:00:30 s	0.083	0.078	0.072	0.073	0.072	0.070	0.068	0.058	0.050	0.041	0.036	0.033
0:01:00 s	0.097	0.095	0.088	0.089	0.088	0.085	0.079	0.067	0.055	0.045	0.037	0.033
0:01:30 s	0.111	0.112	0.103	0.106	0.104	0.099	0.091	0.075	0.060	0.048	0.039	0.034
0:02:00 s	0.125	0.129	0.119	0.123	0.120	0.113	0.102	0.084	0.066	0.051	0.041	0.035
0:02:30 s	0.140	0.146	0.135	0.140	0.136	0.127	0.113	0.092	0.070	0.053	0.042	0.036
0:03:00 s	0.156	0.163	0.152	0.157	0.152	0.141	0.124	0.099	0.075	0.056	0.043	0.036
0:03:30 s	0.170	0.180	0.169	0.174	0.168	0.154	0.135	0.107	0.080	0.059	0.045	0.037
0:04:00 s	0.186	0.198	0.186	0.191	0.184	0.168	0.146	0.114	0.084	0.060	0.046	0.038

Well data tables report each well in a column. The number of wells that can be successfully included depends on the number of digits in the results and the paper orientation (portrait or landscape).

Well Analysis Results Table

This is a sample Well Analysis Results table

Well	Mean V [420]	Y Intercept [420]	R-Squared [420]
A1	31.303	0.066	1.000
A2	34.192	0.066	1.000
A3	33.153	0.061	0.999
A4	31.025	0.075	0.998
A5	26.315	0.091	0.994
A6	19.799	0.104	0.983
A7	11.606	0.116	0.936

Well Analysis results tables are generally easy to include, i.e. all wells can be safely selected, because each well is reported in a row.

Edit Report Items

Protocol > Report Builder

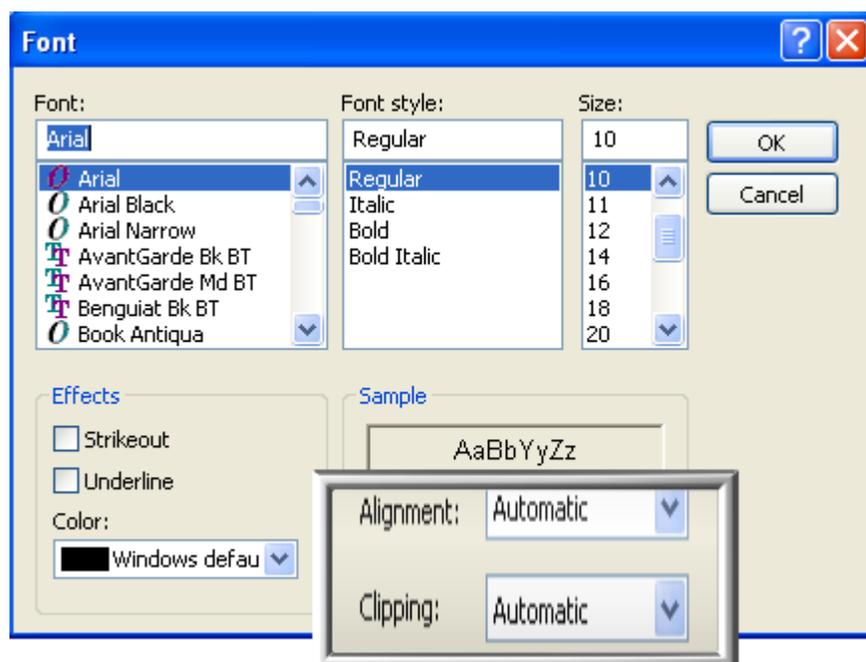
In the **Report Builder**, double click an item in the **Report Content** window to edit its format, font and other attributes specific to the selected item. The editing options available are context sensitive, depending on the attributes of the selected item: matrix, table, or graph.

Change the Font

Clicking the 3-dot button in the **Font** column of a Data View or Report Builder **Edit** screen leads to a standard Windows® Font selector (watch the Report Builder Tutorial for a demonstration):

- In **Data Views**, highlight the item and click **Edit**
- In **Report Builder**, add the item to the **Report Content**, then double click to open it in **Edit** mode

Selecting the **Font**, **Style**, **Size**, and **Effects** is the same as any Windows program. The fonts available for selection are set by your PC's operating system.



Select method for displaying extra-long text items:

In the lower-right corner of the Font screen, use the drop-down lists:

- **Clipping** to select the way to format data points that have too many characters to fit completely in a report or table column and in the cells of a Matrix:
 - **None** fits as much text as possible, using the selected **Alignment** method

- **text...** fits as much text as possible and truncates it using the ellipsis
- **###** replaces all the content with the pound sign or hash mark
- **c:\...\file.ext** only applies to path and filename content, it fits as much text as possible beginning with the filename and extension and progressively truncates the directory's path
- **Alignment** to define the text alignment: left, center, right

❖ Font settings are not retained by the **File Export Builder** because they are not supported in the resulting text file

Fields and Field Groups

For reporting and exporting data, Gen5™ automatically provides data fields and field groups based on inherent information. You can create your own field groups, as well.

- [Learn About Field Groups](#) (below)
- [Create New Field Groups](#) on page 337
- [Assign Fields to Reports](#) on page 340

About Fields and Field Groups

For reporting and exporting purposes, Gen5™ provides data fields based on inherent information. You can create your own **Text field**, as well, to add custom information to a report header or to create a report title, for example.

The responses to **Runtime Prompts**, the steps in the **Procedure**, and the **Reading Date and Time** (logged by Gen5 your computer's calendar and clock) are examples of the numerous data points Gen5 turns into **Fields** that can be added to a **Field Group** for reporting or inserted in a report header or footer. One field group is installed with Gen5: an empty **Field Group** is available in the Data Views, and the report and export builders. You can modify this Field Group and/or create your own in Data Views.

Basic Rules

- **Field Groups** are a collection and arrangement of fields
- Creating and modifying **Field Groups** is done in [Data Views](#); however, field groups can be modified for individual instances after they are included in Report or Export content
- **Field Groups** can be included in Reports and Export files and **Fields** can be added to report Headers and Footers in the [Report Builder](#)
- Once **Field Groups** are added to reports and export content their properties are fixed. Changes made to field groups only take effect going forward, i.e. when you edit a field group in Data Views, the changes are NOT applied to those field groups that are already included in Report and Export Content. You must Remove the original and Add the updated field group in the Report Builder, Power Export Builder and File Export Builder to capture the changes.
- Field Categories include **Text** for user-created information/text and **Blank** for use when editing a field group, header or footer to remove a field.
- A Field Categories reference table is provided on page 338

Creating and Modifying Field Groups

To create a new field group:

1.  Open **Data Views (Protocol>Data Views)**
2. Highlight the **Field Group** label at the bottom of the tree and click **New**
3. Enter a **Name** for the field group in the Edit dialog. Follow the instructions below to define the content of the group.

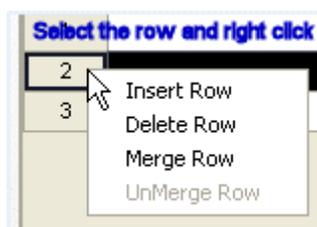
To modify a field group:

1. Open **Data Views**
2. Highlight the **Field Group** you want to modify and click **Edit**. Follow the instructions below to define the content of the group.

❖ You can also **Edit** a field group in the **Report Builder**, but your changes will only apply to the Report for the current experiment. They will not be reflected in the Data Views, will not be available for Export, and will not be applied to any future experiments based on this protocol. Keep this in mind when you're formatting a field group — in most cases it is more efficient to work in Data Views than in Report Builder.

To define the content of a field group:

1. Use the **Add Row** or **Add Column** buttons as needed to set up the desired number of rows and columns.
2. Click in a cell in the table to enable a 3-dot button that leads to the **Fields** dialog to select and format the content of the field. Click the **3-dot** button, and select the **Category** and **Field** you want to add to the group.



3. You can insert, delete, and merge rows, and insert and delete columns using a right-click: select the row or column by clicking its number, then right-click for a menu.



Another shortcut: you can drag and drop rows and columns to change their position. Click in the column/row you want to move until the pointer changes to drag and drop mode, drag it into place, watching for the **red** location indicator, and release to drop it into the desired position.

Field Categories Reference

Category	Field	Hint
Text	User-defined Text	Use this option to define your own report titles
Experiment Information	Experiment File Name	.xpt file must be saved to have a file and path name -Plates per Assay is only applicable to multi-plate protocols.
	Experiment Path Name	
	Plates per Assay	
Procedure	Read step and related data, examples:	These fields are created by the steps you define in the StepWise Procedure. If there is more than one Read step the fields will be numbered accordingly, i.e. every read-related field will be numbered to correspond with its read step. When multiple Kinetic loops are defined, they are similarly numbered. Plate size reports the number of columns x rows
	Plate Type	
	Reading Zone	
	Kinetic Interval	
	Measurement #	
	Sensitivity	
	Temperature	
	Step 1 Description	
Protocol Options	Protocol Type	
	Std Dev Weighting	
Plate Information	Experiment-specific details, examples:	Reports user inputs at read time
	Reading Date and Time	
	Plate ID	
	Runtime Prompts	
	Barcode	
	Reader Serial Number	
	Reader Basecode	
	Plate Comment	
Printing Options	Print Date/Time	Especially useful for Headers or Footers
	Page Number	

System Information	User Name	User information is only useful for Gen5 Secure users Software version reports Gen5's version number
	User Group	
	Software version	
Blank	Empty	Use to delete a previously-added field

Creating a Header and Footer

You can set up a default header and footer that will be applied to all reports, and/or you can create a header and footer for each protocol individually. (Modify the header/footer for individual experiments to override the Default Protocol settings.)

- To assign headers and footers to the Default Protocol (for all newly-created protocols/experiments), select **System > Preferences > Default Protocol Setup**
- To create headers and footers in a Protocol/Experiment (only for the current protocol/experiment): in the menu tree, select **Protocol > Report Builder**

To create a header or footer:

1. In the Report Builder, select the appropriate tab: **Header** or **Footer**
2. Use the **Add Row** or **Add Column** buttons as needed to set up the desired layout
3. Click in a cell in the table to enable a **3-dot** button that leads to the **Fields** dialog to select and format the content of the field
4. You can insert, delete, and merge rows, and insert and delete columns using a right-click: select the row or column by clicking its number, then right click for a menu
5. As needed, select the option to "**use custom header/footer on first page**" to add another tab to this dialog. If unselected, all pages of the report will have the same header and footer.



Another shortcut: you can drag and drop rows and columns to change their position. Click in the number of the column/row you want to move until the pointer changes to drag and drop mode, drag it to the new place, watching for the **red** location indicator, and release to drop it into the desired position. Note: when a row has been merged, columns cannot be moved.

Assigning Fields to Reports

Fields can be used in two ways:

- Used in a header or footer when defining reports, see [Creating Headers and Footers](#) (described above)
- Used to define a Field Group to include in a report or export file

In Report or Export Builder:

1.  In any of the **Header** or **Footer** tabs **or** after adding a **Field Group** to the Report or Export Content and opening it to [Edit](#), click in a cell in the table to enable a 3-dot button, and click it.
2. In the **Category** field, use the drop-down list to select either Text or a category.
3. In the **Field** field, use the drop-down list to select a field, unless you selected Text.
4. In the **Label** field, enter the text you want included in the report. You can customize the label by typing over the default text or remove the label by deleting the text.
5. Use the 3-dot buttons at the **Font** and **Format** fields (if applicable) to modify the default settings. You can replace the default content for the field **Value** the same way:
 - Use the Font dialog to define the **Alignment** of the content in the field: left, center, right. And, if there's a chance the text will overrun the length of the field, choose a **Clipping** format to replace the missing text. Your choices are not displayed in the header or footer table, use Print Preview to assess the effects.
6. Click **OK** to save and apply your choices.

Chapter 16

Exporting Results

Learn about Gen5's numerous data-exporting options in this chapter. You must have **Microsoft Excel 2000** or higher installed on your computer to run the **Quick Export** and **Power Export** features. If you do not have Excel your options are limited to the **File Export** or **Right-Click** options.

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Exporting Results

Gen5 provides these exporting tools:

-  **Quick Export:** to instantly export the current view to a **Microsoft® Excel** worksheet
- **Power Export:** to export selected data to **Microsoft Excel**
- **File Export:** to export selected data, excluding curves, to a text file (for use in another software application)
- **Right-Click Menu Options - Copy to Clipboard and Save As:** to copy or save the current selection for use in another software application

Prerequisites

For the Quick Export and Power Export features, you must have **Microsoft Excel 2000** or higher installed on your PC. Use the File Export or Right-Click options if you do not have Excel.

About the Export Tools:

- The **Power Export** and **File Export** methods require selecting the content you want included in the output file **before** executing the export for a designated plate
- You can **save** your export selections with the **Protocol**, to reuse them every time you run an experiment based on that protocol
- Exporting data is like generating a report, it is done individually for each plate*. While you can select the export content in a protocol, you must run (or execute) the export in an **Experiment** (after selecting a plate or multiple plates)
- In an experiment, to run the export, you can select a plate in the menu tree and **right click** for a menu that offers the **Power Export** and **File Export** options
[* except in multi-plate assays: refer to the [Multi-Plate Protocol](#) chapter]

 To preview the export-file output on-screen before generating an export file, use the [Report Builder](#) and **Print Preview**: add the same elements to a report as you do to the export, then click Print Preview to view the results online.

Export Multiple Plates to One File

When you run multiple plates in an experiment you can export all the data to one text file:

1. In the menu tree, select/highlight multiple plates (by holding down the Ctrl key)
2. Right-click and select **File Export**

❖ Make sure the **File Export Settings** are defined to automatically append the data

Quick Export



Click the **Quick Export** button to instantly create a copy of the current screen in Excel®.

Where it works:

Wherever you see the **Quick Export** button, you can export the view. And almost every Data View has a Quick Export button. For example, the Matrix, Statistics, Cutoff and Validation tabs offer it and the Graph tab has two, one for the curve and another for the Curve Results Table.

If you don't have MS Excel:

Use one of these options to select content and export it for use in another software application:

- **File Export Builder:** to export selected data to a text file (see page 346)
- **Right-Click Menu Options - Copy to Clipboard and Save As:** to copy or save the current selection for use in another software application (see page 346)



Quick Export Settings

Protocol > Protocol Options > Export Options > Quick Export Settings
System > Preferences > Default Protocol > Protocol Options > Export Options > Quick Export Settings

You can define default settings for Quick Export, which can be overridden in individual protocols. Use the controls in the **Default Protocol** to base all new Protocols on them. Override the default settings in individual protocols using the **Protocol Options**.

- ❖ **Important: Default Protocol settings** are applied "going forward," they do not override existing protocol settings. They effect only newly created protocols.

Export Row and Column Headers

Select this option to include the Matrix and Statistic table row and column headers with the data exported to Excel™

Text Options

- **Formatted Text:** exports text as defined in Gen5, retaining the selected font and any customizations
- **Text Only:** exports text as characters only, without an associated font or any customization performed in Gen5

Excel Target

Select a method for positioning the exported content in Excel:

- **New Workbook and Target Cell:** opens a new workbook and aligns the content starting at the target cell
 - **New Worksheet and Target Cell:** creates a new worksheet (within a workbook) and aligns the content starting at the target cell
 - **Current Worksheet:** adds content to the current worksheet
Gen5 will launch Excel with a new worksheet if necessary. When multiple Excel sessions are open, Gen5 will prompt you to select a worksheet before performing the Quick Export.
 - **Target Cell:** aligns content beginning at the target cell, fill the Target Cell field when you select this option
 - **Current Selection:** places content beginning at the currently selected cell
 - **Append to Bottom:** places content beginning at the next available row
- Ask me when I export:** This option lets you determine the **Excel Target** every time you use Quick Export. Gen5 will prompt you for a placement option prior to executing the **Quick Export**.

Right-Click Menu Options

In addition to the **Quick Export** option available in most views, Gen5 offers three other features for "exporting" the current view for use in other software applications.

 Simply right-click to select the entire current view for a quick output. Or, to limit the output: click and drag to select contiguous cells or hold down the Ctrl key and click to select noncontiguous cells, then right-click for an option.

Copy to Clipboard



1.260	1.360	1.460	1.560
1.660	1.760	1.860	1.960
2.060	2.160	2.260	2.360

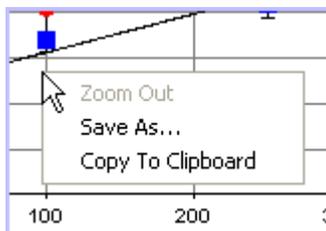
1. **Right-click** in almost any view and select the **Copy to Clipboard** feature
2. Open another software application, e.g. Microsoft® Word, WordPad, Paint, Outlook and similar products offered by other manufacturers, where you want to use the Gen5 content
3. **Paste** the selected content. Try:
 - **Ctrl-V**
 - Right-click and select **Paste**
 - From the menu, select **Edit> Paste**

Quick Print

After selecting specific content or a current view, right-click and select the QuickPrint feature.

It is similar to printing from the Report Builder, you can select a local printer. The current view or selected content is printed in formatted text with row and column headers.

Save As



In any graph, i.e. Curve or Well Zoom, you can:

1. **Right-click** and select the **Save As** feature

2. Gen5 opens the standard Save As dialog so you can browse to any file/directory available to your computer to choose the **Save In** location



3. Use the drop-down list to select the **Save as type**:
 - Portable Network Graphic (.png)
 - 24-bit Bitmap (.bmp)
 - CompuServe Graphics Interchange Format (.gif)
 - JPEG Format (.jpg)
 - Windows Enhanced Meta File (.emf)

Export to File (File Export Builder)

This option creates a text (.txt) file of the selected content for use in another software application.

Protocol > File Export Builder

Using the File Export Builder

Select the Content to Export

Highlight items in the **Available Data Views** and **Add** them to the **Export Content** box:

- You can [Drag and Drop](#) items into the Export Content box
- Drag and drop to change the sequence order of export items in the output file
- Change the **format** and **data sets** associated with an item in the Export Content box: Highlight an item and double click, or right click and select **Edit**
- Highlight an item and click **Remove**, to remove it from selection
- **In multiple-read (multi-index read) protocols**, like kinetic analysis, it is necessary to select a range of reads or a range of wells to include in an export item. Certain data elements, like Well Data, Well Results, and Well Zoom, **require manual selection** of the specific wells to include. Except for area scan results, you can select multiple wells for simultaneous reporting in the resulting table or graph. For details see [Reporting Well Analysis Results](#) in the (previous) Reporting Results chapter

 **Gen5** opens report items in **Edit** mode when a selection is required. For example, when you select a Matrix item to report a multi-index/kinetic read, you must select a Range of read numbers. One matrix or grid will be reported for each read number.

❖ **Limitation: "Curves" and "Scan" data sets**, created from kinetic analysis and area scans, cannot be exported with this feature. If you have Excel®, use the Quick or Power Export options.

Export Multiple Plates to One File

When you run multiple plates in an experiment you can export all the data to one text file. After defining the export content, (and making sure the File Export Settings do not prevent it):

1. In the menu tree, select/highlight multiple plates (by holding down the Ctrl key)

2. Right-click and select File Export

You can also use the [File Export Settings](#) to do this automatically:

- make sure the plates do not result in unique filenames,
- set the "prompt before saving" option to **Never** and **Append**

❖ **** Items marked with asterisks indicate 1 of 3 conditions:**

1. You must [select](#) specific data points from a very large (multi-read) data set **or**
2. The experiment did not generate the expected results, an error occurred **or**
3. The Procedure and/or Data Reduction steps that generated the item have been changed making it invalid ******

Using the Default Protocol

You can define default settings for File Export, which can be overridden in individual protocols:

- Use the controls in the [Default Protocol](#) to base all new Protocols on them.
- Override and refine the default settings in individual protocols using the **File Export Builder**

❖ Content options in the Default Protocol may be limited by the lack of a defined Procedure.

❖ **Important: Default Protocol Settings** are applied "going forward," they do not affect existing protocols. They are applied only to newly-created protocols.

File Export Settings

Protocol> Protocol Options> Export Options> File Export Settings
System>Preferences>Default Protocol>Protocol Options>Export Options>
File Export Settings

You can define default settings for File Export, which can be overridden in individual protocols:

- Use the controls in the **Default Protocol** to base all new Protocols on them
- Override the default settings in individual protocols

File Naming Convention

Set up a naming convention to apply to the export file:

- **File Name:** use the text field to build a naming convention. You can put any combination of text and Gen5-provided data points in the field to become the name applied to consecutively-saved files:

-  Click the options arrow at the end of the field to select from the Gen5-provided data points.
- Add or replace default text with your own text
- Notice the underscores Gen5 places between data points, they can be retained or removed according to your preferences. Generally, it is good practice to use filenames without spaces.
- **Example:** Gen5 displays an example of the file name you create

❖ The filename must comply with Microsoft® filename conventions, e.g. it must not contain so-called offending characters: \ / : # ? " < > |

Separator

Export files contain "delimited" data, i.e. data separated by a user-defined symbol or character. Select or enter the desired Separator using the buttons for **Tab**, **semicolon**, **comma** or **Other**. If Other, enter the symbol or character in the text field.

File Location

Specify the location for **Saved** files:

- **Last folder used:** puts the Excel file in the folder last used by Gen5's File Export engine
- **Folder:** select an existing folder or define a name to apply to a newly-created folder
 -  Use the **3-dot** button to browse to the desired location for file storage
 -  Use the options arrow to name a newly-created folder using the Gen5-provided data points.
 - You can add text for naming a newly-created folder, if desired
 - **Example:** Gen5 displays an example of the folder name you create

When Exporting, prompt before saving file:

- **Always** prompt users by opening the **Save As** screen whenever they export a plate file, allowing users to alter the file name and location on-the-fly
- **Only if the file already exists:** open the Save As screen for saving export files only if Gen5 generates a filename that already exists
- Never, if it the file already exists: never open the Save As screen
- **Append:** add this plate's data to the bottom of the existing file
- **Overwrite:** replace the existing data with this plate's data

Include:

Use the checkboxes to include any of the items offered:

- **Headings:** includes the Name of selected data elements as a section heading in the export file (just like the section headings in a Gen5 report)
- **Matrix ...:** includes the well location column and row labels, e.g. A-H and 1-12
- **Statistics ...:** includes column headers of the tables, e.g. Well ID, Name, Well, Conc/Dil.

Power Export

Prerequisite

You must have **Microsoft® Excel 2000** or higher installed on your PC to use Power Export.

Power Export Explained

Gen5 joins forces with Microsoft Excel to give you high-powered results reporting. Gen5 compiles all the data and customizations made in an experiment and exports it to Excel, along with a Gen5 toolbar.

In Excel, you design the report using the Gen5 toolbar to select the desired experiment content. Then you can use Excel's native tools to perform complex calculations and customize the report's appearance.

Using Power Export is a two step process: first, ideally when setting up a protocol, you select the content for export. Second, after running an experiment, you execute the Power Export for each plate.

Whenever you select a Power Export option, Gen5 launches Excel. Initially, to select the content of the export, which inserts placeholders in the worksheet for the data to be generated in the experiment. Later, when you execute Power Export in the experiment, the selected data fills the placeholders.

With Power Export you can:

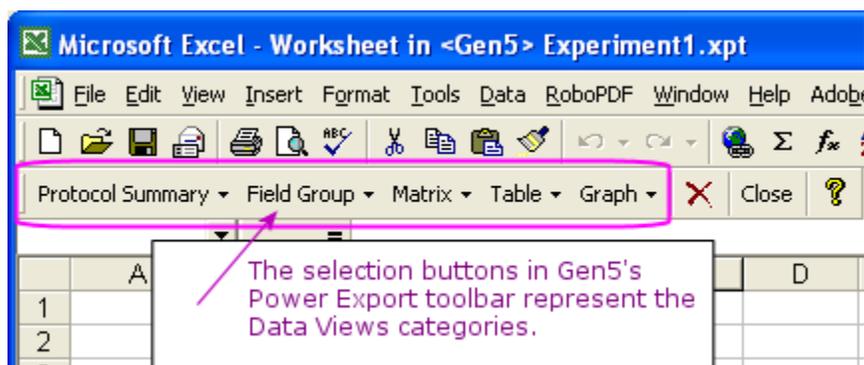
- Take full advantage of Excel's document customization features to generate publication-quality reports
- Include pictures, drawings, and company logos in reports
- Create custom formulas in Excel to perform additional calculations on the Gen5 experiment data
- Take advantage of Excel's charting capabilities to create bar graphs, pie charts, etc.

 **Before** defining the Power Export, customize the **Data View** elements in Gen5 that you'll include in the Excel report. While, you can make changes to Gen5's data elements when using Power Export in Excel, it is faster and easier to make the bulk of your choices first in Gen5.

❖ Watch the Tutorial for a demonstration on how to use Power Export

About the Power Export Toolbar

Gen5's **Power Export Builder** launches **Excel**® with a custom toolbar:



The selection buttons of the toolbar mirror the categories in [Data Views](#) (except Protocol Summary, which is only offered in the reporting tools). Use the buttons to select the content you want included in the Power Export. When you make a selection, Gen5 puts a "Results Object" or placeholder for the information in the Excel worksheet. The placeholder is filled in with results data (after the plate is read) during Power Export execution.

❖ In **Office 2007** the Power Export Toolbar is placed in the **Add-Ins** tab/ribbon, which is added to the default ribbons when Excel is launched by the Power Export Builder.

- **Protocol Summary** = two pre-defined listings of the Procedure and Data Reduction steps defined in the protocol
- **Matrix** = a grid that represents the microplate
- **Table** = a columnar presentation of the data
- **Graph** = a standard curve or well zoom generated from a Curve or Well Analysis
- **Field Groups** = user-selected or defined data points useful for reports

Power Export Execution

When you execute the Power Export in an experiment (i.e. highlight a plate in the menu tree, right click and select **Power Export**), Gen5 launches Excel with the data from the experiment filling in the placeholders selected with the Builder. You can run Excel as you normally do. The connection between Gen5 and Excel is severed. To modify a report element use the **Power Export Builder**.

 **BioTek** recommends customizing the Data View elements in Gen5 before selecting them for the Excel report. You can make changes to Gen5's data elements using the Power Export toolbar in Excel, but it is faster and easier to make the bulk of your choices in Gen5 first.

How to use Power Export

1. When setting up the protocol, after customizing the Data Views, select **Power Export Builder** from the Protocol menu tree. This launches Excel with **Gen5's Power Export Toolbar**.

❖ In **Office 2007** the Power Export Toolbar is placed in the **Add-Ins** tab/ribbon, which is added to the default ribbons when Excel is launched by the Power Export Builder.

2. In **Excel**, use the **Power Export toolbar** to select the content you want to import from each plate (described below).
3. Define the **Power Export Settings** (page 355).
4. When you run an experiment based on the protocol, highlight a plate in the menu tree, right click and select **Power Export**.

Whenever you select Power Export, Gen5 launches Excel. Initially, to select the export content, inserting placeholders for the data in the Excel worksheet. When you run Power Export in the experiment, the selected data fills the placeholders. Then, you can use Excel's native toolset to prepare the report for publication.

❖ To modify a report element you must use **Power Export Builder**.

💡 Do NOT "protect" the worksheet in Excel when building the report with Power Export Builder; that is do not engage Excel's Tools>Protection settings.

Using the Power Export Toolbar in Excel



Gen5's **Power Export Builder** launches **Excel**® with a custom toolbar for selecting content for the export.

How to select content:

1. Select the starting cell in the Excel worksheet where you want the Gen5 content to begin.
2. Click the down arrow of a selection button: **Protocol Summary**, **Field group**, **Matrix**, **Table**, or **Graph** on the Power Export toolbar and select an item. Gen5 presents a Selection/Data/Format dialog specific to the selected item.
3. Make selections and/or modifications to the data format, as required for the item. Just like Customizing Gen5 Data Views and Reports. You can limit the Range of data points or change the Format/Font, as needed.

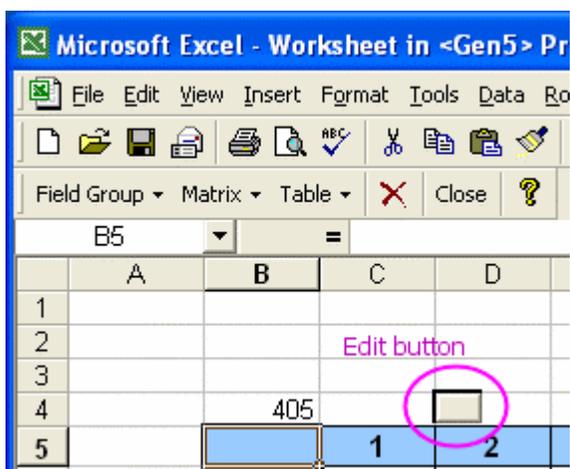
❖ Certain data elements, like Well Data, Well Results, and Well Zoom, **require manual selection** of the specific wells to include in the export. Except for Area Scans, you can select multiple wells for simultaneous reporting in the resulting table or graph.

- Repeat steps 1-3 to select all the specific data items you want included in the Power Export, filling the worksheet with the required placeholders.
- Click  to return to the Gen5 workspace.

Now you're ready to execute **Power Export** in an experiment: highlight a plate in the menu tree, right click and select **Power Export**.

How to modify selected content:

When you select content for the Power Export, Gen5 puts a button above each data view in the Excel spreadsheet, right next to the starting cell for the content.



Click the button to modify the selected content, i.e. open it in **Edit** mode

Running Power Export

Highlight the plate in the menu tree > right click > Power Export

- After you've selected the content to export using the **Power Export Builder**
- And read the plates in an experiment



Highlight the plate in the menu tree and click the **Power Export** button on the toolbar or right click and select Power Export. Gen5 launches Excel® with the selected content. You can then use Excel as normal.

Power Export Settings

**Protocol > Protocol Options > Export Options > Power Export Settings
System > Preferences > Default Protocol > Protocol Options > Export Options > Power Export Settings**

You can define default settings for Power Export, which can be overridden in individual protocols. Use the controls in the **Default Protocol** to base all new Protocols on them. Override the default setting in individual protocols using the **Protocol Options**.

Select method:

Save after Export: select this option to enable Gen5's file naming and saving routine, then define your preferences below. They will be executed when the Excel file is saved. When this option is not selected, Power Export performs as expected but does not save or name the resulting file.

Close after Export: select this option to run **Power Export** in the background. This feature creates, saves, and closes the Excel file using the defined settings after obtaining the content selected with the [Power Export Builder](#). If you do not select this option, Gen5 will keep Excel open until you close it.

File Naming Convention

Set up a naming convention to apply to the Excel files created with Power Export:

- **File Name:** use the text field to build a naming convention. You can put any combination of text and Gen5-provided data points in the field to become the name applied to consecutively-saved Excel files:
 -  Click the options arrow at the end of the field to select from the Gen5-provided data points
 - Add or replace default text with your own text
 - Notice the underscores Gen5 places between data points, they can be retained or removed according to your preferences. Generally, it is good practice to use filenames without spaces.
 - **Example:** Gen5 displays an example of the file name you create

❖ The filename must comply with Microsoft® filename conventions, e.g. it must not contain so-called offending characters: \ / : # ? " < > |

File Location

Specify the location for **Saved** files:

- **Last folder used:** puts the Excel file in the folder last used by the Power Export engine
- **Folder:** select an existing folder or define a name to apply to a newly-created folder
 -  Use the **3-dot** button to browse to the desired location for file storage
 -  Use the options arrow to name a newly-created folder using the Gen5-provided data points

- You can add text for naming a newly-created folder, if desired
- **Example:** Gen5 displays an example of the path and folder name based on your input

When Exporting,

Prompt the user to confirm the path and filename by showing Excel's Save dialog:

- **Always:** whenever the save occurs
- **Only if the file already exists:** when the same name is applied to an existing file
- **Never (overwrite the file if it already exists):** do not show the Save dialog, replace the existing file with the current one when a file with the same name exists

Export Row and Column Headers

Select this option to include the Matrix and Statistic table row and column headers with the data exported to Excel™

Text Options

- **Formatted Text:** exports text as defined in Gen5, retaining the selected font and any customizations
- **Text Only:** exports text as characters only, without an associated font or any customization performed in Gen5

❖ **Important: Default Protocol settings** are applied "going forward," they do not override existing protocol settings. They effect only newly created protocols.

Chapter 17

Managing Files

This chapter offers instructions and suggestions for managing your Gen5 experiment and protocol files, with a focus on Gen5's database. Methods for recovering from Database Errors are provided.

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Managing Files

Gen5 offers two methods for storing its protocol (.prt) and experiment (.xpt) files. You can use a secure database called **SharedDB** (and its companion [LocalDB](#)) provided by Gen5 or you can use the Windows® File System. Both options let you set up Gen5 files to be shared by multiple users. Conversely, you can prohibit file sharing by only installing Gen5 locally, on an individual's PC.

The SharedDB ships with Gen5, and for all software levels, it contains security information and the Plate Types Database. Except for Gen5 Secure, the File Storage option must be changed to use the database for storing .prt and .xpt files.

Gen5 Secure is intended to be used with the SharedDB, it is a major component of making it "secure." In Gen5 Secure the SharedDB contains user accounts and a System audit trail, in addition to the security information and the Plate Type database, and it is set up by default to store .xpt and .prt files and their associated audit trails.

How to manage files

Gen5 provides [Database Management](#) tools when its Shared database (SharedDB) is used for file storage. The SharedDB can be moved to a shared, network directory, where all required users can access it. When the Windows file system is used for file storage, you manage files using Windows Explorer. In addition to storing the files online, files can be stored on CD or diskette.

File Storage

System > Preferences > File Storage Mode

Use this control to select a method for storing protocol and experiment files.

❖ **Attention Gen5 Secure users:** To ensure 21 CFR Part 11 compliance retain the setting to use the **Gen5 Database**

About File Storage

Gen5 provides two methods for storing protocol and experiment files. You can use the secure, shared-access database provided with Gen5, which is required for compliance with the FDA's [21 CFR Part 11](#) regulation on electronic records submission. Alternatively, you can use the file system provided with the Windows® operating system on a local computer or network (LAN). If your organization is unconcerned with FDA regulations, the choice is a matter of preference. However, one advantage to using the Gen5 Database is its ability to recover from a system crash. New and modified files are saved as [Temporary Files](#) in the database and can be used to recover information that wasn't saved before a system failure.

❖ **Clarity™** Luminometer protocol files, with a .bpf extension, cannot be stored in Gen5's shared database. They are typically stored in the C:\Program Files\BioTek\Clarity\protocols folder.

How to

- 🕒 Select an option for storing Experiment and Protocol files:
 - **Gen5 Database:** all actions related to managing and maintaining files, like File>Open, File>Save, Browse..., and so on, will occur in Gen5's SharedDB.
 - **Windows File System:** Gen5 will not control the management of files. Actions related to managing and manipulating files will be determined by the Windows operating system, e.g. you can use Windows® Explorer. Generally during file management activities like File>Open, Windows begins at the last directory and folder used.

❖ **AutoSave Feature:** Gen5 offers this feature to give you additional control over the storage of saved files.

Database Management

Organize Your Database Files

- ❖ During regular installation, **Gen5 Secure** installs and enables the shared database to store experiment and protocol files. **All other levels of Gen5** must elect to use the database at **System> Preferences> File Storage**

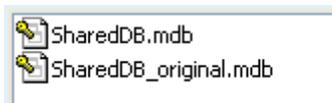
All of your file management requirements can be fulfilled using Gen5's secure databases. You'll be most satisfied with the final structure if you spend some time planning it up-front. In a multiple-user environment, you can set up Gen5's database on a shared-network drive (LAN) so multiple users can access the same protocol and experiment files, including the Default Protocol.

Multiple Databases: You can create multiple copies of the clean, installed SharedDB, renaming them with meaningful titles for use by various projects or teams or researchers. Within each database you can set up a consistent file structure, e.g. specific folders for specific types of Protocols and Experiments, or a different folder for each user. The possibilities are endless.

Backups: Performing backups on a regular schedule is highly recommended to preserve your data. And, Gen5 provides a tool to schedule backups to occur periodically. See below.

File Management Recommendations

- Put a copy of the **SharedDB on a shared-network drive** where all your Gen5 users can access it. Be sure to set each user's [Database Configuration](#) to point to the correct location.
- **Before [moving the SharedDB](#)** to a network location, make a copy of it to use as a template for future use:
 1. In the [default SharedDB folder](#), highlight the original, right-click and select **Copy**
 2. De-select the original (click elsewhere in the dialog), right-click and select **Paste**
 3. Highlight the copy, right-click and select **Rename**
 4. Give the copy a unique name, like SharedDB_original.mdb



- Consider **setting up shared databases** for different projects or teams within your organization. You can follow the steps defined above to create multiple databases in the same folder (or directory), or you can move the unique databases to a different network location/folder. Use Database Configuration to point user's Gen5 sessions to the correct database.

- **Regularly archive and backup** the database to preserve your records. There are numerous ways to do this, so BioTek recommends following your organization's existing policy for securing data. For example, if you put the shared database on the network and your network is backed up every night, this may be sufficient. You can use Gen5's Optimize and Backup Settings to facilitate your data-protection policy.
- Consider using Gen5's automatic [Save](#) feature to create a new, date-stamped folder for storing experiment records. This is an especially good practice for large labs with multiple users who run hundreds of plates per day. Gen5 will keep all that data organized by date. Define this kind of file management setting in the Default Protocol so it will apply to all newly-created protocols (**System>Preferences>Default Protocol>Protocol Options>Save**).
- Gen5 handles multiple, simultaneous users performing database management tasks by giving precedence to the user with the greater administrative rights.

About Gen5 Databases

All levels of Gen5 install two databases during regular installation: SharedDB and LocalDB. Only **Gen5 Secure** is initially set up to use the Gen5 Database for experiment and protocol file storage. All other levels of Gen5 must elect to use the database to store experiment and protocol files at **System> Preferences> File Storage**

- **SharedDB** can be set up on a network for sharing information amongst multiple users. It contains all protocol and experiment data files and their associated audit trails, the plate types, and reader-diagnostic history data. In **Gen5 Secure**, SharedDB also contains security settings, user accounts, and a system audit trail for shared events. This database **can** be moved, renamed, and copied. So, if desired, you can create a unique database for individual projects, teams, or other classification.
- **LocalDB** contains the local setup information, including the Reader Configuration. For **Gen5 Secure**, this database also contains an audit trail for local events. LocalDB is stored on the computer's hard drive, and it **cannot** be moved or renamed.
- Default database location: During normal installation, Gen5 installs its databases:
 - **Windows XP and 2000** systems: C:\Documents and Settings\All Users\Application Data\BioTek Instruments\Gen5 (*software edition*)*(version #)*\SharedDB or LocalDB
 - **Windows Vista:** Windows XP and 2000 operating systems: C:\Program Data\BioTek \Gen5 (*software edition*)*(version #)*\SharedDB or LocalDB

❖ You may need to change your operating system settings to view the Application Data folder. In Windows® Explorer, select Tools>Folder Options>View and make sure it is set to "Show hidden files and folders."

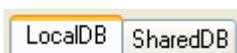
- **Max Size:** the maximum size of the database files is 2 gigabytes (Gb). At startup, Gen5 checks the remaining size of the database. Warning messages are displayed when the database size exceeds 1536Mb. Use Gen5's [maintenance](#) and backup features to archive your database records.
- **Gen5 has built-in error recovery** modes, when your connection to the database is lost for any reason, Gen5 saves any unsaved files as **Temporary Files**. After a system failure, the next time you open an affected protocol or experiment file, Gen5 offers to replace the unsaved files with the Temporary Files. Say **Yes** to recover any changes made to the files before the system failure, say **No** to open the files as they were last saved, before the unsaved changes were made. Newly-created files are saved as Temporary Files, also. Following a system failure, you can rename these temporary files with the proper filename extension (.xpt or .prt) using Gen5's **Maintain Files** controls.
- **File locking:** When a file is opened in Gen5 it is “locked” to protect it from being modified (saved or renamed) by a different user. When a second user attempts to open the file, they will receive a message stating: “File <filename> is already in use. Do you want to open it in read-only mode?”
- **Gen5 offers automatic backup:** you can define settings for regularly and automatically backing up and optimizing databases with Gen5's **Auto-Optimize** feature.

Database Configuration

System > Database Configuration

Prerequisite

Some features of this dialog require System Administrator privileges. Contact your System Administrator if you are unable to perform actions as expected.



When Gen5 is installed, there are two primary databases, a shared and a local database. They are named **SharedDB.mdb** and **LocalDB.mdb** respectively. The LocalDB cannot be moved or renamed. The SharedDB can be moved and renamed. All operations affecting them take place using their respective tabs, except the upgrade utility.

Troubleshooting

When launched, Gen5 attempts to connect to the Gen5 databases. Review this information if errors occur: [Database Errors](#) (page 369)



Gen5 presents the **Reset Connection** button only when it detects an error that can be repaired by its functionality. Click it, several times if needed, until it is grayed-out. Review the [Database Errors](#) information if the button doesn't fix the error.

Source



Gen5 displays the current location of the database.

- **LocalDB:** You cannot move or rename the LocalDB database, thus the 3-dot button is disabled. If necessary to see the full pathname of its location, you can click inside the text field and scroll to the right.
- **SharedDB:** Click the 3-dot button to view the current location of the database. You can move, rename or copy the **SharedDB** database. Learn how in the [Getting Started Guide](#) shipped with the product CD.

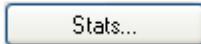
Test



Use the **Test** button to check the connection to the Gen5 database.

- Potential error messages are referenced in the Troubleshooting section above.

Stats



Use the **Stats** button to assess the number of files and amount of space used in the Gen5 database.

- Generally, the most important information is **Size**: reported as used/available. If the used value is nearing the available value, it's time to move some files. Use the DB [maintenance tools](#) to archive files not currently being used.
- Occasionally using the **Optimize** option (described below) helps keep the DB in good shape.

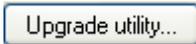
Optimize



Click the **Optimize** button to engage Gen5's corruption-repair and size-compacting functions. **Learn more in Gen5's Help.**

Periodically Optimize and Backup: to direct Gen5 to automatically backup and optimize the database on a regular basis. Select the option and define its settings. Use the 3-dot button to modify the settings. (See page 367)

Upgrade



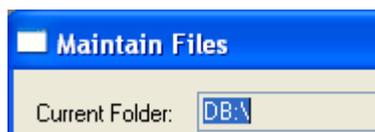
There are two uses for the Database Upgrade Utility:

- install an updated version of the software
- install a higher level of software, e.g. upgrade Gen5 to Gen5 Secure

Maintaining Files

System > Maintain Files

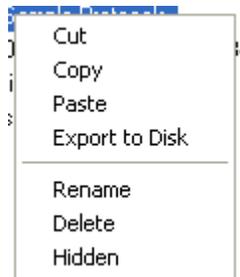
Use this dialog as you would Windows® Explorer to manage your Protocol (.prt) and Experiment (.xpt) files. **Gen5 Secure** tracks activities, like creating, moving, and deleting files, in its System Audit Trail.



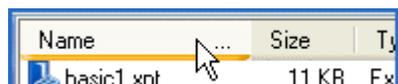
Important: the DB (database) this dialog opens is controlled by **Database Configuration**. It is the **SharedDB** defined as **Source**.

Tools:

-  Toggle the view between **Details** and **Icons** of the files and folders. In **Details** view, click on a column header to set the sort order. For example, click Type to organize the files by file type, or click Modified (once or twice) to sort them in the desired ascending or descending date order.
-  **Create a new folder** to save certain files separately
-  **Refresh** or update the view to show files or folders added by another user
-  **Move up** one folder/directory level with this button



- Right-click menu:** highlight one or more items (folders or files) and right click for a pop-up menu. Notice the **Hidden** option: system administrators can hide/reveal selected files to prevent other system users from accessing them. Warning for Windows XP users...
- Ctrl+C** to copy, **Ctrl+V** to paste, and the **Delete** key are also supported
-  Highlight a file or folder and **Delete** it with this button. You must delete sub-folders (i.e. folders within a folder) before you can delete their parent or higher-level folder



- Column Headers** can be used to sort the files: click on a header to sort the files in ascending/descending order by that category. For example, click Modified to sort the files by "last modified" date. Click the same column header to reverse the order.

Tasks:

Find step-by-step instructions for performing these tasks in Gen5's Help:

- Organize your files
- Copy to CD, diskette or other portable media
- Export a file
- Import a file
- Reduce the database size

Optimize and Backup Database

System > Database Configuration

Optimize Now

Optimize

Click the **Optimize** button to run Gen5's database compacting and backup program.



Before "optimizing" the database, close all protocol or experiment files.

When to do it:

- After restoring database connections following an error
- After exporting and deleting records to reduce the database size (Maintaining Files)
- After system audit trail events are exported or deleted

❖ Gen5 creates a backup copy of the database (in its present location unless a different storage location was selected in the Optimize Periodically settings) before beginning optimization. It is named: <original filename>_yymmdd_hhmmss.mdb (year, month, day_hour, minute, second). .mdb is the filename extension. Backup files can be used to repair Gen5 when the current database file is corrupt, for example.

Periodic Optimization

Optimize the database periodically

Use this option to schedule Gen5 to conduct Optimization on a regular basis. BioTek suggests once per week.

Use these controls to define the rules for regular, automatic database optimization.

❖ **Important:** Only use this automatic method for backing up files when you're saving them to a network or external drive. Do not use it to save a backup to the same (local) hard drive used to store the original database. Manually backup your database if you are limited to one hard drive/PC, unconnected to a network.

How it works:

Whenever Gen5 is launched it checks the contents of the backup location to determine if optimization is due. When it is, Gen5:

1. Renames and moves a copy of the current database to the **Backup** location. The naming convention is:
Auto_Backup_<original filename>_yymmdd_hhmmss.mdb.
2. Executes the repair and compact operations on the current database
3. As needed, it deletes the oldest archived (or previously backed-up) database file to correspond to the current settings
4. Gen5 displays a status gauge on screen to tell users the operation is underway

How to define the settings:

1. Set the number of days to run the optimization in the **Optimize every _ days** field
2. **Backup:** you can retain Gen5's default location for storing a backup copy of the database, or click the **3-dot** button and select an alternative location.
3. Define the number of previously backed-up or archived database files to keep in the backup location in the **Keep _ last archived databases** field

Database Errors

Certain conditions can cause database-related errors:

- **The database file is not available.** Potential causes: the shared database is on a network and the cable is unplugged, or the file is locked by another user
- **The database file is corrupt.** Potential cause: an incomplete write operation occurred because Gen5 was closed unexpectedly due to a power outage or hardware problem
- **Verification of the database capacity failed.** Potential cause: the maximum size of the database (2 gigabytes) has been exceeded
- **File importation failure.** Potential causes: the filename extension is wrong, a protocol file was misnamed with an .xpt filename extension instead of .prt

Fixing the errors

1. First, make sure non-Gen5-system issues are resolved, for example:
 - network cables are plugged in and the network is up and running;
 - another Gen5 user is **not** currently performing database maintenance routines;
 - the [Database Configuration](#) for both the SharedDB and LocalDB databases point to the expected locations;
2. Then, follow these instructions for fixing the errors:
 - Fixing a Database Connection Error (page 369)
 - Fixing a Corrupted File Error (page 370)
 - Fixing a Database Capacity Error (page 371)
 - Fixing a File Importation Error (page 371)
3. If the above solutions do not work, try **Restoring an archived database** (page 372).

Fixing a Database Connection Error

 Connection to SharedDB failed.

This type of error is most commonly caused by a network timeout or disconnection, or corruption to the SharedDB file residing on a network.

To fix:

1. Make sure you are properly connected to your network, if applicable, i.e. cables/wires installed and the network server is live.

2. Click **Reset Connection** at the Database Configuration screen. Gen5 immediately opens the Database Configuration screen after displaying the error message. Otherwise, select **System > Database Configuration**

- ❖ **Important:** Click the **Reset** button several times, if needed, until it is grayed out.
- ❖ **Quit:** If Gen5 cannot restore the network connection, usually because of external factors, e.g. the network is down, when you click **Quit** at a secondary error message, Gen5 will save any currently opened and modified files to the [Windows Temp](#) directory. When the database is restored you can import the files from the Temp folder to the database.

3. When the database connection has been repaired, click **Optimize**.

If Reset button fails:

If your network is performing as expected, and you've checked the cabling from your PC to the network, and the Reset button fails to re-establish a connection with the shared database:

1. Reboot your PC

If the same message is displayed:

2. **Restore a previous version of the database** or contact your System Administrator to inspect and repair your system connection.

Fixing a Corrupted File Error

 Database file is corrupt

There are two potential ways to fix a corruption error:

- **Reboot your PC** to try to clear the error by restarting the system.
- **Install an archived version** of the LocalDB.mdb: use Windows® Explorer to locate and restore a backup of the database.

- ❖ Windows® Explorer provides an option to hide certain folders from view, if you cannot find the **Application Data** folder (C:\Documents and Settings\All Users\Application Data) it is probably hidden. In Explorer, select Tools>Folder Options>View and enable Show hidden files and folders

If these options fail to repair Gen5's behavior, contact BioTek TAC.

Fixing a Database Capacity Error

When the cumulative size of the files in your database nears or exceeds its capacity (2 gigabytes) Gen5 displays an error message or warning. You must reduce the size of the database. There are numerous ways to accomplish this, the least invasive method, to remove files, is described here.

Prerequisite

This function is only available to the **System Administrator**. You must login, **System>Administrator Login**, as the Administrator to access these controls.

- ❖ **Optimize** the database before proceeding to see if Gen5 can compact the database sufficiently to comply with its size limits. Select **System>Database Configuration>Optimize**. When the process is finished click the **Stats...** button to check the current size.

To reduce the size of your database:

1. Select **System> Maintain Files**
2. Highlight multiple database records: hold down the **Ctrl** key while selecting records
3. Right click and select **Export to Disk** from the pop-up menu
4. **Select a folder** (using the standard Windows dialog) where you want to save or archive the Gen5 records
5.  Back in the **Maintain Files** dialog, highlight the same records, and click the **Delete** button (or right click and select Delete)
6. Finally, **Optimize** the database to ensure it's running in top form.

Fixing a File Importation Error

-  File importation failure
-  The requested file may be a Protocol file
-  The requested file may be an Experiment file

Gen5 tests the file's format when you perform certain operations like importing a file and **File>Open**. One of these error messages may be displayed when an incompatibility is found.

Change the filename extension to fix these errors

1. Locate the offending file: depending on the current **File Storage** mode:
 - using the Gen5 Database, select **System> Maintain Files**
 - using the Windows File System, use **Windows® Explorer**

2. Highlight the file, right click and select **Rename**:
 - change the filename extension for a misnamed Protocol file from .xpt to **.prt**
 - change the filename extension for a misnamed Experiment file from .prt to **.xpt**
3. Retry the desired action, e.g. open the file, import the file

Restore an archived database

When Gen5's error recovery processes cannot resolve database errors, a final-resort solution is to replace the current database file with an archived or backup copy of it. Ideally, you or your System Administrator has regularly (or at least recently) backed up or archived the database. Gen5's Optimize tools can perform backups regularly, for instance.

 Check the [Auto-Optimize Settings](#), if they were defined, to determine the location of the last-saved backup file.

How to

1. Using Windows® Explorer, locate the last-saved archived or backed-up database file.
2. **Copy** and **Paste** it to the [desired location](#). **Rename** the database file, too. (All of these commands are available from the right-click menu.)
3. In Gen5, select **System > Database Configuration**
4. Select the **SharedDB** tab
5.  Next to the **Source** field, click the 3-dot button and browse to the location selected in Step 2.
6. Click **OK**.

Chapter 18

Security

Primarily for Gen5 Secure users, this chapter describes Gen5's tools for managing security issues, including audit trails, electronic signatures and user accounts.

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Security

To meet the FDA's electronic records requirements, **Gen5 Secure** offers several tools to enable a secure software environment. In addition to the content provided here, you can find detailed information about these features in the Gen5 Help:

- [Audit Trail](#) on page 381
- [Electronic Signatures](#) on page 379
- [System Administrator's "To Do List"](#) on page 8
- Manage User Accounts:
 - [Setting Up New Users](#) on page 388
 - [Changing a User's Passwords](#) on page 375
 - [Changing a User's Privileges](#) on page 391
- [About 21 CFR Part 11](#) on page 378

Changing Your Password

❖ For Gen5™ Secure Only

System > Security > Users

Users other than the System Administrator are limited to changing their own login password.

How to change your password:

1. Select **System > Security > Users**
2. Identify and open your user account: highlight and click **Edit** (or double click)
3. Enter your current password in the **Current Password** field
4. Enter your new password in both the **New** and **Confirm** password fields
5. Click **OK**.

The password will take effect the next time you log into Gen5.

❖ Contact your System Administrator if you've forgotten your password. He/she can change your password without knowing the current one.

Login/Password Controls

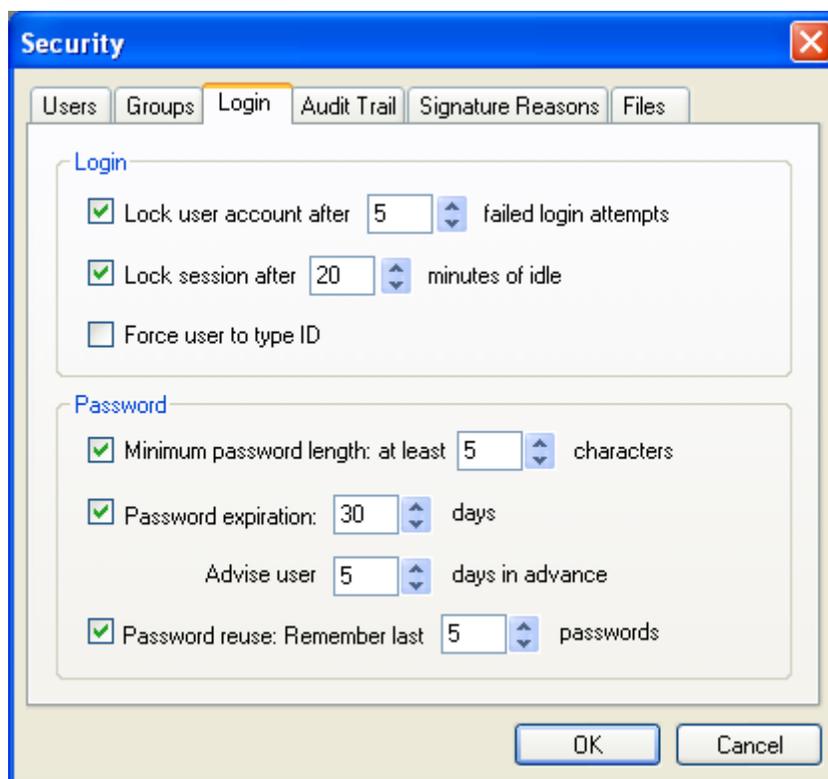
❖ For Gen5™ Secure Only

System > Security > Login

Prerequisite

Only the **System Administrator** can access these controls. You must login: **System > Login/Logout**, as the Administrator to change the settings.

❖ **Important:** The default settings shipped with Gen5 Secure, and shown in the screenshot below, comply with the [FDA's 21 CFR Part 11](#) requirements (page 378) on controls for identification passwords.



Login

- **Lock user account after:** Specify the number of successive failed login attempts a user may make before being locked out of Gen5. This feature does not apply to System Administrator accounts and only a System Administrator can [reinstate a locked out account](#). Valid entry range: **2-10**. When this feature is unchecked, users login attempts are unlimited. Compliance with 21 CFR Part 11 requires setting a limit for failed login attempts.
- **Lock session after:** Specify the number of minutes that a Gen5 session can be idle before it is locked and requires successful user login to reactivate. A

session is considered idle when there is no keyboard or mouse activity and Gen5 is not controlling a reader activity. Valid entry range: **1-1440** minutes. Compliance with 21 CFR Part 11 requires setting an idle-time limit.

- **Force user to type ID:** apply this control if your security rules require users to enter their ID at login and to apply their [Signature](#). When this feature is unchecked, the last user's ID is displayed in the login and signature screens and users can select an ID from a drop-down list of users. This is not a requirement for compliance with 21 CFR Part 11.

Password

- **Minimum password length:** Specify the minimum number of alphanumeric characters required for a valid password. Valid entry range: **2-10** characters.
- **Password expiration:** Specify the number of days a password can be used before users are required to change it. When users let their password expire without changing it, their accounts are locked out and only a System Administrator can [reinstate a locked out account](#). Valid entry range: **1-10000** days. If this feature is unchecked passwords do not expire. Compliance with 21 CFR Part 11 requires an expiration period.

❖ **Lock out:** when a user's password has expired, the system administrator has two choices:
 = manually remove the Locked out flag: this resets the password expiration period allowing the user to login using his/her current password.
 = enter a new password for the user (which unlocks the account) and tell the user to login with the password you have assigned him/her. Advise the user to [change the password](#) after logging in.

- **Advise user:** If password expiration is set, specify the number of days before their password expires to alert users to change their password. Valid entry range: **1-30** days, but cannot exceed the number of days to Password Expiration.
- **Password reuse:** Specify the number of passwords Gen5 will remember for each user's account to prevent a recently used password from being reused. Valid entry range: **2-20**.

FDA's 21 CFR Part 11

This is a description of the FDA's electronic-records submission requirements and how they are satisfied by Gen5™ Secure.

A significant component of this secure software environment is the ability to create individual user accounts to ensure that only authorized users can gain access to the system and to any restricted functions. A site-designated System Administrator creates and maintains the user accounts.

Gen5 Secure complies with FDA's Electronic Signatures rule, 21 CFR Part 11:

- **System Administrator** — The System Administrator creates and maintains user accounts and user groups to specify which Gen5 functions shall be protected from use by limited-access users, e.g. masking data. The System Administrator sets special password and login characteristics including minimum password length, password aging, and idle session time-out. The System Administrator is also responsible for managing the database of Gen5 files and audit trails.
- **Support for Multiple Users** — Each user is identified by a unique combination of User ID and encrypted password. Users must log in to Gen5 Secure with these identifiers to gain access.
- **Time-stamped Audit Trails** — Activities such as user login/logout, protocol and experiment creation and modification, and plate reading are permanently logged in a secure database.
- **Embedded Signatures** — Authorized users can electronically sign protocol and experiment files. Electronic signatures are permanent and remain a part of the overall data record for the life of that file.
- **Secure Record Storage** — Gen5 proprietary files (.prt and .xpt) are stored in a secure shared-access database. Activities performed on files within this database (such as rename, move, copy, overwrite, and delete) are performed within the Gen5 environment, and every change is tracked in an audit trail. In addition, the System Administrator can configure the system so that these activities may only be performed by Power Users or high-level users.
- **Protected Functions** — The System Administrator can protect a variety of functions from use by limited-access users, e.g. Standard Users. These functions include the deletion, renaming, modification, and overwriting of various record types.

Signing Protocols

❖ For Gen5™ Secure Only

System > Security > Signature Reasons

Gen5 Secure provides users the ability to sign (i.e. sign off on) a protocol or experiment file. Your System Administrator can define the Signature Reasons to give meaning to each signature recorded. Gen5 ships with three reasons: **Authorship**, **Review**, and **Approval**. These terms can be kept, modified, or added to.

When users create, review, or perform any other activity on a Protocol or Experiment for which a signature or sign-off is required, they use the **Sign** option, select a **Reason**, and enter their password to confirm the action.

Representatives can sign off on files for another user. Gen5 provides an option for **two users** to digitally sign records as representatives of a third user.



What do you want to do:

- [Create/Modify Signature Reasons](#) (below)
- [Sign a Protocol or Experiment](#) (page 380)
- [Include Signatures in the Experiment Report:](#)

To include Signatures in a report or export file:

- 1 Open the preferred reporting tool: Report Builder, File Export Builder or Power Export Builder
- 2 In the **Available Data Views** or Excel report objects, find **Table**, and add **Signatures** to the report content.

Gen5 reports the Signatory, Reason, and Date

Signature Reasons

❖ For Gen5™ Secure Only

System > Security > Signature Reasons

Prerequisite:

This function is only available to the **System Administrator**. You must login: **System > Login/Logout** as the Administrator to access this control

How to create/modify Signature Reasons:

Simply, click in the text fields of the **Signature Reasons** table to add new or replace existing reasons for signing protocols and experiments.



Sign

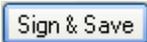
Protocol > Sign

Plate > Sign

To sign off on a **Protocol**, select **Sign** from the Protocol menu or click the **Sign** button

- To sign off on an **Experiment**, highlight a plate in the menu tree, select **Sign** from the Plate menu (or right click and select **Sign**, or click the **Sign** button)

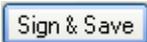
Sign Off on a file:

1. Use the appropriate option (described above) to open the signature screen
2.  **Reason:** Use the drop-down list to select the reason for signing the file. Your System Administrator creates and maintains the Reasons selection list .
3. **User:** Gen5 sets this to match the user who is currently logged in. Use the drop-down list to change it, if necessary.
4. **Password:** Enter your password.
5. Click 

If the file hasn't already been saved, Gen5 opens the **Save** dialog.

Sign as a Representative:

Two users are required to sign as a Representative of another user. Follow steps 1 and 2 above, then:

1. Select the checkbox for **Representative of** and use the drop-down list to identify the user being represented.
Gen5 adds 2 tabs to the dialog, one for each representative.
2. Each representative must select their user ID and enter their password.
3. Click 

If the file hasn't already been saved, Gen5 opens the **Save** dialog.

Audit Trail

About Audit Trails

Depending on the level of Gen5 you're running, one or more Audit Trails keep track of certain activities and build a "change history" log of events. The protocol and data audit trails cannot be edited or deleted. They are part of the permanent record of the protocol or experiment file.

When the [Audit Trail Notification](#) feature is deployed in **Gen5 Secure**, users are prompted to enter comments into the record each time a logging event takes place. You can view and report the audit trail and calculation warning logs, as needed.

Audit Trail Types:

- **Data Audit Trail** — *All levels of Gen5* — logs values-masking and -editing events, plate addition and deletions, and other related events. Data audit trails occur at the Experiment level to log the experiment-related events, like which protocol it was based on, and at the Plate level for plate-related events, like the read's status/progress
- **Protocol Audit Trail** — *Gen5 Secure Only* — logs all events related to the creation and modification of protocol files, like changes to plate layout and data reduction steps
- **System Audit Trail** — *Gen5 Secure Only* — logs system-level events, like user login/logout and reader-setting modifications, maintenance and updates to database folders and files, and so on. System events can be archived and deleted from the database.

All the audit trails include an Event description, a time and date stamp, **Gen5 Secure** logs the ID of the user logged in at the time of the event, and any user-entered comments

Audit Trail Notification Options

❖ For Gen5™ Secure Only

System > Security > Audit Trail

Prerequisite

Only the **System Administrator** can access these controls. You must login: System>Login/Logout, as the Administrator to change the settings.

About Audit Trail Notification

These controls turn on or off notification to users when an audit-trail-logging event occurs. When Notification occurs, users are provided an opportunity to add a **Comment** to the record. If the system-generated text does not provide as much detail

about the event as you'd like, you can use the Comments feature to encourage users to enter more useful information. For example, when the plate layout of a protocol is changed, Gen5 simply logs the event as "Plate Layout changed", while the user can add details to the record like, "Added blanks: H11, H12."

Notification and comment control for each type of audit trail can be selected individually using the checkboxes and drop-down lists.

How to

1. **Turn on Notification:** for each type of audit trail using the checkbox to turn on or off (remove the checkmark) notification of an event. Notification identifies the event with a brief description and provides a text area for users' **Comments**
2. **Prompt user for a comment:** if Notification for an audit trail is turned on, select a comment-required setting:
 - **Accept an empty comment:** user is not required to enter text in the Comment area
 - **Invite user to type a comment:** if user does not enter a comment during notification of an event, the **Message inviting the user to enter a comment:** defined in the text box below, will be presented to them. You can replace the default text with your own wording
 - **Comment required:** if user does not enter a comment during notification of an event, the **Message requiring the user to enter a comment:** defined in the second text box below, will be presented to them. You can replace the default text with your own wording. Users will not be permitted to quit/exit the audit trail notification without entering some text in the Comment area

Exporting Audit Trail Events

❖ For Gen5™ Secure Only

System > Audit Trail > Export button

Use this feature to export System Audit Trail events for archiving and to generate a report of the events. Exported records can be deleted from the system during the export process to maintain the size of the database, and to remove unnecessary records.

❖ **Important:** Gen5 does not retain control over the exported text files. It is your organization's responsibility to ensure the security of the exported audit trail records.

To export audit trail events:

1. Select **System > Audit Trail**
2. Click the **Shared** or **Local** tab to select the source database of the records you want to export
3. Click **Export**
4. Enter a **date range** of the events you want included in the export file
5. Check or ignore the **Delete events after exportation** option
6. Set the **Save in** field to the location you want to send the text file to. Use the standard Windows® browse tools to select the location
7. Click **Save** to export the events

After export, compact the database:

If the Delete events after exportation option was used, the System Administrator should:

1. Make sure there are no users are currently logged into to the shared database
2. Select **System > Database Configuration**
3. In the Shared or Local database, as appropriate, click **Optimize**

 After exporting the file, you can open it with Notepad® or a word processor, to review or print it.

Data Audit Trail

Each plate and each experiment has a Data Audit Trail, a change history or log of automatically-recorded **events**. An event is an action like:

- Plate read started
- Plate successfully read
- Plate read failed with message "Reader error: 0IE0"
- Modify Value - Well A2 - 390 - Old: 2.180 - New: 2.080

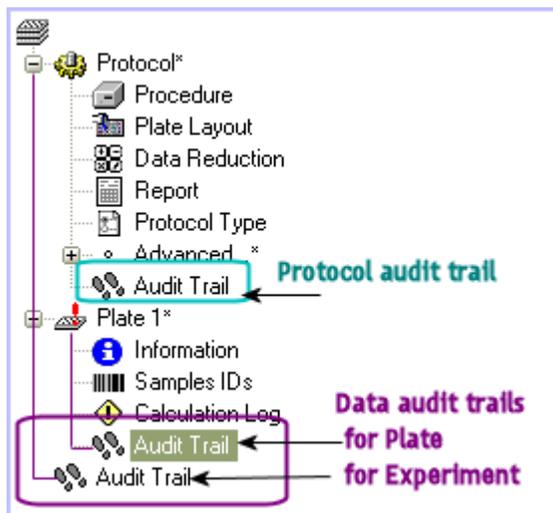
❖ When values are masked or edited, Gen5 logs the event as Mask or Modify Value <Well>-<Data set name><read index for kinetic/scan><position for scan>; old and new values are provided when the value is changed.

What events are logged:

The level of software determines the types of events logged by the data audit trail:

- **Gen5:** All Gen5 levels log data changing/masking and plate addition and deletion events, and plate-read status and warnings
- **Gen5 Secure** also logs digital signature events
- All audit trails contain a description of the Event, a time and date stamp. **Gen5 Secure** also contains the ID of the user logged in at the time and any user-entered comments (if [Audit Trail Notification](#) is turned on)

Viewing Audit Trails:



In the menu tree, each Plate has a Data Audit Trail, and so does the Experiment
You can also open them from a menu:

Plate>Audit Trail

File>Audit Trail (for the Experiment)

Reporting/Printing Audit Trails:

You can include the Data Audit Trail in reports and export files, and copy and paste items to an external file:

- In the **Report Builder** and **Export Builder**, look for Audit Trail under Table in the Available Data Views tree, to add an item to the Report or Export Content
- **Copy and Paste:** In the audit trail screen, highlight event details and use **Ctrl+C** to copy. Open Notepad or a word processor, and paste it with **Ctrl+V**

Security:

Data audit trails cannot be edited or deleted. They remain a permanent part of the Experiment file for the life of that file.

Protocol Audit Trail

❖ For Gen5™ Secure Only

Each protocol has an audit trail that automatically logs all events related to its creation and modification. For example:

- Copy of protocol X (the full path and filename)
- Plate Layout modified
- Data Reduction modified

What events are logged:

- Procedure
- Data Reduction steps
- Plate Layout
- Report and Export definitions
- Miscellaneous protocol options
- A description of the Event, a time and date stamp, the ID of the user logged in at the time (if applicable) and any user-entered comments (if [Audit Trail Notification](#) is turned on)

Viewing the Audit Trail:

- In the menu tree, expand the Protocol branch to locate its Audit Trail
- In a multi-plate calibrator or single-assay protocol, the Protocol Audit Trail is split into a main log and separate Method Audit Trails for each Calibration Plate and Other Plates.

Reporting/Printing the Audit Trail:

- In the **Report Builder** and **Export Builder**, look for Audit Trail under Table in the Available Data Views tree, to add an item to the Report or Export Content

- In the audit trail screen, highlight event details and use **Ctrl+C** to copy. Open Notepad® or a word processor, and paste it with **Ctrl+V**

Security:

Protocol audit trails cannot be edited or deleted. They remain a permanent part of the Protocol and/or Experiment file for the life of that file.

System Audit Trail

❖ For Gen5™ Secure Only

System> Audit Trail

System-level events, like user login/logout and reader-setting modifications, are automatically recorded in the System Audit Trail. For example:

- Logout (computer and user ID)
- File "database + filename" created
- File "database + filename" pasted from clipboard

What events are logged:

- User login and logout
- System security updates (user accounts, login and password parameters, protected functions, audit trail notifications, and file location and format)
- Plate Type Database modifications
- System Test and Test Plate runs, and adding and modifying Test Plates
- Reader settings changes (reader type, probe selection, communication parameters, and filters/wavelengths)
- System setup changes (protocol defaults, format settings, start-up options, and database settings)
- User-customizable application settings (such as toolbar and position/size of main window)
- A description of the Event, a time and date stamp, the ID of the user logged in at the time and any user-entered comments (if [Audit Trail Notification](#) is turned on)

Viewing the System Audit Trail:

Select **System>Audit Trail** to open the viewer.

Reporting the System Audit Trail:

You must **export** Audit Trail Events to print them from a text or word processing file.

Exporting Audit Trail Events:

Gen5 provides a quick and easy method for exporting the System Audit Trail. Use this feature to establish a regular schedule for "archiving" past events and to generate a report of the events, if needed. The export action itself is logged in the audit trail. Exported records can be optionally deleted from the system.

❖ **Important:** Gen5 does not retain control over the exported text files. It is your organization's responsibility to ensure the security of the exported audit trail records.

User Accounts

About User Accounts

❖ For Gen5™ Secure Only

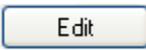
System > Security > Users

Prerequisite

This function is only available to the **System Administrator**. You must login: **System > Login/Logout** as the Administrator to access all the controls. Non-administrators are limited to changing their own password and selecting a Startup Action and Protocol Folder.

How to Create, Modify or Delete User Accounts

Only an Administrator can add, modify, or delete users. Except for the Administrator, any user account can be changed or deleted:

-  Click **New** to set up a new user
-  (Double-click or) Highlight a user and click **Edit** to modify its name, password, or Group assignment
- Highlight a user and click **Delete** to remove the user account

Setting User's Permissions

Gen5 Secure: System > Security > Users > Edit

All other Gen5 levels: System > User Setup

Prerequisite

This function is only available to the **System Administrator**. You must login, **System > Administrator Login**, as the Administrator to access these controls

Depending on the level of software, User's are given permission to perform tasks based on their **Group** assignment in **Gen5 Secure** or the **User Permissions** given to all users in all other levels of Gen5.

Gen5 Secure

User Permissions are defined by the **User Group**. When you select or change a user's Group assignment you're simultaneously assigning their permissions:

1. Select **Security > Users**
2. Highlight the user and click **Edit** (or double click the user record)
3. Use the drop-down list to change the **Group** assignment

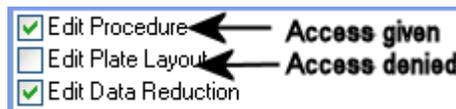
❖ See [Modifying User Permissions](#) (on page 391) to change the permissions assigned to a group

Gen5, Gen5 ELISA, Gen5 Reader Control

For all levels of Gen5, except for Gen5 Secure, there are only two types of users: System Administrator and User (non-administrator). The System Administrator can set or change the User Permissions for non-administrators. Gen5 provides all user rights and privileges to administrators.

To change User Permissions:

1. Login as the System Administrator
2. Select **Security > User Permissions**
3. Add or remove a tick mark for each [permission](#) to give or deny access to it to all non-administrator users



About User Groups

❖ For Gen5™ Secure Only

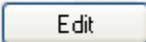
Gen5 Secure uses **Groups** to manage the rights or permissions granted to users. When creating (or maintaining) a group, you define the level of access and the controls available to certain types of users, and then assign actual users to the groups. Gen5 ships with three groups: **Administrator**, **Power User**, and **Standard User**.

The System Administrator and Power User groups are given access rights to all functions. The Administrator's rights cannot be changed, and include additional rights to manage user accounts that are not extended to Power Users. When Gen5 Secure is installed, the Standard User is limited to the following permissions. The System Administrator can change these controls as needed:

- Quick Read/Use Default Protocol
- Add a New Plate
- Create/Edit Sample IDs
- Edit Plate Information
- Edit Report Builder
- Create folder in database

How to create new and modify existing groups:

Only a System Administrator can add, modify, or delete groups. Except for the Administrator group, any group can be changed or deleted, and any group can be renamed.

-  Click **New** to set up a new group
-  Highlight a group and click **Edit** to modify its name and permissions
-  Highlight a group and click **Delete** to remove it as an option. First you must reassign any users to another group. You cannot delete a group with users assigned to it.

Creating/Maintaining User Accounts

❖ For Gen5™ Secure Only

System > Security > Users

Prerequisite

Most options for user accounts are only available to the **System Administrator**. Non-administrators are limited to changing their own password and selecting a Startup Action and Protocol Folder.

User ID

Enter a unique ID using 1 to 16 alphanumeric characters. The user will enter or select this ID when logging into Gen5 and when signing files.

Full Name

Enter the user's name. This name will be associated with events logged by this user's actions and with the digital signature applied by this user.

Group

Choose a Group membership to assign access rights and permissions to the user. Users receive the rights assigned to the Group.

Status

The checkbox shows whether or not the user's account is currently locked. The System Administrator can lock or unlock the account. When a user's account is locked, the user cannot log into Gen5 and cannot sign files. A user's account may become locked due to one of three events:

- Intentional lock by the Administrator through this dialog
- Automatic lock if the user exceeded the number of successive failed login attempts
- Automatic lock if the user's password expired

❖ See important information about expired passwords on page 377.

Startup Action

Use the drop-down to select the preferred method for starting Gen5:

- **Startup Window** is the default setting, it offers several options including creating a new item or opening a recently used item
- **Create new experiment** opens Gen5 with the Protocol selection dialog open, as if the user had selected File>New Experiment
- **Start at main menu** opens Gen5 showing the File, System and Help menus only. Since neither a protocol or experiment is open, the workspace is blank.

Protocol and Experiment Folders

Browse to or enter the full path and directory to define the folder in which the current user will typically store protocol and experiment files. If a folder is not specified, Gen5 will default to the most recently-accessed folder.

Password

Assign a password for the user to enter the first time he/she logs in to Gen5. Instruct users to change their password after the first login using the Password you've assigned. Users can only change their own password. System Administrators can change any user's password.

Modifying User Permissions in Gen5 Secure

❖ For Gen5™ Secure Only

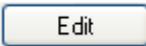
System> Security> Groups> Edit

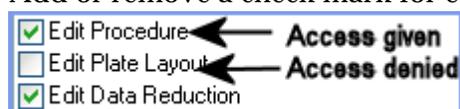
User's are given permission to perform tasks based on their **Group** assignment in **Gen5 Secure**.

Prerequisite

This function is only available to the **System Administrator**. You must login, **System>Login/Logout**, as the Administrator to access these controls

How to Change User Permissions:

1.  Select **System> Security> Groups**, highlight the group and click **Edit** to change the permissions or access rights of group members. Or click **New** to create a new user group.
2. Add or remove a check mark for each function to grant access or deny it.



Denying user permissions usually results in making the applicable dialog **Read Only**.

Permissions

Protocol/Experiment Controls

This table describes the capability each Permission gives users. The Audit Trail icon shows when Gen5 Secure logs the activity in the Protocol or Data Audit Trails. Links are provided to learn more about the function.

Function	Audit Trail
Create a new Protocol: Access to menu options File>New Protocol, File>Save As, and Startup Page options to create a new protocol file	
Open a Protocol: Access to File>Open, Recent Files List and Startup Page options to open an existing protocol	
Perform a Quick Read/Use default protocol: Access to creating an experiment from the default protocol	
Add a New Plate: Access to menu options and toolbar buttons for Adding one or multiple plates to an experiment	
Delete a plate: Access to Plate menu option to Delete, i.e. remove the plate information and all data associated with the plate (if any) from an experiment	
Create/Edit Sample IDs: Access to Plate menu and menu tree options to enter or modify Sample IDs for each plate or in a Batch for multiple plates	
Edit Plate Information: Access to Plate menu and menu tree options to modify the Plate Information. Note: Information is intended to be captured at run-time, for each plate in an experiment	
Mask/Unmask values: Access to Mask button in data views to select individual wells and mark them to be ignored in data reduction and curve plotting	
Edit values: Access to Change button in data views to select individual wells and change/enter alternative data for use in curve plotting and data reduction	
Re-read plate: Access to Read button after plate has been read to overwrite the current measurement results with newly acquired measurements	
Simulate Read: Access to Simulate option of the Plate Read dialog to let Gen5 simulate a reading instead of actually reading the plate. (Useful for Gen5 training/tutorials.)	
Read from File (import): Access to Read From File option of the Plate Read dialog to acquire/import reading data from a text file	

Enter Manually (raw data): Access to Enter Manually option of the Plate Read dialog to manually enter (type in) reading data instead of actually reading a plate	
Edit Protocol: This switch gives or denies access to the next nine related functions. You can override it by individually selecting the permissions and assigning access	
Edit Procedure: Access to the Procedure dialog to alter the reading requirements and related events, like Delay, Shake. Gen5 always prohibits users from changing an experiment's Procedure after the first plate is read	
Edit Plate Layout: Access to the Plate Layout dialog to change the plate layout, Well IDs, Concentrations/Dilutions	
Edit Data Reduction: Access to the Data Reduction dialog to change data reduction steps, add new steps or alter existing ones	
Edit Report Builder: Access to the Report Builder to create or modify the report definitions	
Edit Runtime Prompts: Access to create or modify the "prompts" (information requests) presented to users at read time. Users' responses become Plate Information	
Edit Data Views: Access to alter the format/font of data views items; create custom data views	
Edit File Export Builder: Access to select and modify the content for export to a text file; define the filename and format settings for export files	
Edit Power Export Builder: Access to select and modify the content for export to Excel®	
Edit Protocol Options: Access to define miscellaneous, protocol-related parameters	

System Controls

This table describes the capability each Permission gives users. The Audit Trail icon shows when Gen5 Secure logs the activity in the System Audit Trail

Function	Audit Trail
Manage and Maintain Systems: This switch gives or denies access to the next five items. You can override it by individually assigning access to the permissions	
Edit Default Protocol: Access to define or modify the Default Protocol Settings	
Edit file storage mode: Access to menu option System>Preferences>File Storage to alter the option: database or Windows® file system	

<p>Edit Read from File options: Access to menu option System>Preferences>Read from File Settings to alter the designation of the text delimiter for importing data via text files</p>	
<p>Manage and maintain Database: Access to change the location of the local and shared databases, and their backups. Run maintenance tasks and tests, and repair errors. Only when File Storage "uses the SharedDB"</p>	
<p>Delete System Audit Trail Events after export: Access to delete records after exporting them to a text file. All users can export records, only users with this permission are able to delete them</p>	
<p>Manage and Maintain Devices: This switch gives or denies access to the next four related permissions. You can override it by individually assigning access to them</p>	
<p>Edit Reader Settings: Access to Reader Configuration to set up and alter the settings. Denying access restricts the user's ability to change a reader's filter/wavelength settings in Gen5</p>	
<p>Edit Plate Types: Access to create, modify or delete records in the Plate Type Database</p>	
<p>Define Universal Plates: Access to Diagnostics options to set up and modify the Universal Test Plate records used to conduct testing</p>	
<p>Delete Diagnostic Test History: Ability to delete test records. All users can view the test history, only users with this permission can delete the records</p>	
<p>Manage and Maintain File Storage: This switch gives or denies access to the next seven related permissions. You can override it by individually assigning access to them. They are only applicable when File Storage "uses the SharedDB"</p>	
<p>Create folder in Database: Ability to create a new folder while maintaining database files and when saving protocol and experiment files. Users denied this function are limited to saving files in existing database folders</p>	
<p>Delete/Overwrite folder in Database: Ability to delete or overwrite (Save As) folders and files from/in the database</p>	
<p>Export file from Database: When maintaining database files, ability to use the right-click menu to Export to Disk</p>	
<p>Rename folder/file in Database: Ability to rename database files and folders in the database</p>	
<p>Move folder/file in Database: Ability to relocate folders and files within the database</p>	
<p>Import file to Database: Ability to import, paste from clipboard, or drag and drop files from another location</p>	

Chapter 19

Reader Control

This chapter supplements the Getting Started Guide instructions for attaching a reader to Gen5 with more in-depth information about how to control, configure and test your reader.

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When you have Two Readers

Gen5 lets you set up and control two readers simultaneously. Here's how Gen5 determines which reader to deploy when you have two readers attached to your PC.

❖ **Note:** Fluorescence and Luminescence protocols are not instantly interchangeable between Synergy 2 and other BioTek readers. (The Synergy 2 has a newer basecode.) When you try to read a plate using a protocol defined on a different reader, Gen5 will alert you of the need to edit the Read step. To use a protocol created with a different reader, open all the Read steps while communicating with the current reader. This action will update the protocol to match the current reader's capabilities.

Compatibility

Gen5 tests the readers for compatibility with the **Procedure** when you:

- Edit/Open an Existing Procedure
 - Read a plate
1. All available, i.e. not busy, readers are tested for compatibility. A reader performing a read is considered busy.

Compatibility is determined by these criteria:

- If the protocol was created for the Clarity, then the reader must be a Clarity. If the protocol was not created for the Clarity, then the reader cannot be a Clarity
- If it is not a Clarity protocol, Gen5 tests the reader's capability to perform the required detection method:
 - Absorbance: minimum and maximum wavelengths supported
 - Fluorescence
 - Luminescence
 - Spectrum Scans
 - Linear Scans
 - Area Scans
- Gen5 tests the reader's capability to perform:
 - Incubation: minimum and maximum temperature supported
 - Shake
 - Dispense: number of dispensers available
- Gen5 tests the reader's capability to perform:
 - Well Mode: maximum number of reads, shakes, and dispenses
 - Synchronized Plate Mode: maximum number of synchronized plate reads, shakes, and dispenses

- Pathlength Correction
2. If exactly one reader meets the criteria, it is automatically selected to edit the Procedure or read the plate
 3. If more than one reader meets the criteria, Gen5 determines if one of them was last used to edit the Procedure or read the plate. If so, then it is selected. If not, the user is prompted to select which reader to use.

Gen5 checks the Protocol for a reader type and serial number to determine which reader was last used. If it does not find an exact match, but only one of the readers is the same type, that reader is selected.

4. If none of the readers matches the criteria:
 - Gen5 will display an error message and offer to **Continue** or **Cancel**. Users who select **Continue** can modify the Procedure to make it compatible with their reader

Reader Configuration

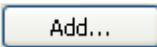
System > Reader Configuration

Use these controls to tell Gen5 about the attached reader(s) and to retrieve software version information for tech support or other purposes. The status of an instrument is displayed as Ready or Busy: Ready to perform a read or Busy doing so.

❖ **Special note for Clarity users:** Configuration parameters and port settings can only be defined through the Clarity PC software. See [Setting up the Clarity Luminometer](#) in the **Getting Started Guide**

Add a Reader

Click the **Add** button to connect up to two readers to the system. You'll select the **Reader Type** and the **Communication Port** its plugged into on your PC (see page 400), and if necessary, define **Setup** properties.

 and  lead to **Reader Settings** on page 403

Modify a Reader

Highlight a reader and click **Modify** (or double click a reader) to change the reader's Com Port or **Setup** properties

Delete a Reader

Highlight a reader and click **Delete** to eliminate it from potential use by Gen5.

ActiveX Version

Click the **Active X** button to retrieve the latest software version number:

BTIReaderInterface Control v0.72.4

You may need to provide this information when seeking assistance from BioTek's TAC (Technical Assistance Center)

Controlling the Clarity

System > Reader Control > Clarity

Microplate Holder Control

The Clarity's microplate holder can be opened and closed only through software control. Under **Move Plate**:

- Click **Out** to extend/open the microplate holder
- Click **In** to retract the microplate holder into the reading and heating chamber

❖ **Important:** Do not forcibly push in or pull out the microplate holder! If for any reason you cannot control the holder using the software, use the Allan key supplied with the instrument to extend or retract the holder. Refer to the Clarity™ Operator's Manual for more information.

Prime

The reagent lines and injectors should be primed with the dispensing fluid before running the protocol. Click **Priming** to open the **Priming Parameters** dialog.

❖ Refer to the Clarity Operator's Manual for complete priming instructions.

To prime the injectors:

1. Fill the reagent bottle(s) with the fluid to be dispensed.
2. Select **System > Reader Control > Clarity** and click **Priming** for the **Priming Parameters** dialog. All installed injectors are displayed. Injectors marked by an asterisk (*) have yet to be initialized. Initialization takes place automatically when the injector is used.
3. Click on the injector's check box. The drop-down list box **Direction**, **Volume [μl]** and **Strokes** are enabled.
4. Set the **Direction To Mpl**. Specify the **Volume [μl]** and the number of **Strokes** or **cycles**. The total liquid volume is displayed as the **Total Volume**.
5. Click **Prime** to prime the injector(s).

Heating

The Clarity's optional incubator is controlled via software. Click the **Incubation...** button to access the **Sample Incubation** dialog.

❖ Refer to the Clarity Operator's Manual for complete heating instructions.

About Com Ports

Com Ports are communication ports that allow your computer (PC) to connect to and control other devices. BioTek ships the required serial and/or USB cables with the reader. You must tell Gen5 which com port is used to connect to a reader. (More on page 403)

Serial cable (see samples on page 401)

Generally, Windows®-compatible PCs have two serial com ports, which it assigns as Com1 and Com2. If you're uncertain which com port the serial cable is plugged into, try Com1 and **Test Communication**. If you receive an error, try Com2.

Advanced users can attach additional com ports to a PC, and can use Window's Control Panel to identify or modify the com port number.

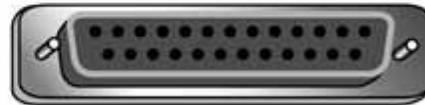
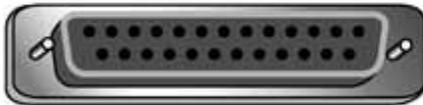
USB cable (see samples on page 402)

For compatible instruments, BioTek ships **USB-Driver** software along with the USB cable. Follow the instructions provided for USB installation, e.g. installing the USB Driver software, and review the [ComPort Guide](#) to learn how to identify or modify the com port number.

Troubleshooting

Review the information provided in the **Troubleshooting** chapter for resolving communication errors.

Serial Communication Cables and Ports 9-pin and 25-pin



USB Communication Cables and Ports



Reader Settings

System > Reader Configuration > Add/View

- ❖ **Prerequisite:** You must be authorized to make changes to the Reader Configuration. If the options are grayed out, contact your System Administrator for access rights.
- ❖ **Special note for Clarity users:** Configuration parameters and port settings can only be defined through the Clarity PC software.

Use these controls to tell Gen5 the type of reader, communications port and baud rate to use:

1.  Click the down arrow to select the Reader Type
2. Enter number of the Com Port in the text field
3. BioTek recommends retaining the default Baud Rate or transmission speed. If you have a compelling reason you can select another rate from the list

- ❖ **Baud rate settings can cause "serial read" errors:** when the baud rate is set to a non-default setting for Synergy and PowerWaveXS readers, Gen5 will be unable to communicate with them if they are turned off and then turned on again while Gen5 is running.

4. Generally, the **Setup** option is **not** needed. Gen5 communicates with the reader to obtain the probe sizes and configuration of the filter/wavelengths tables.

Reader Types

Gen5 only needs to know the basic model of reader, then Gen5 can communicate with the reader to learn its specific capabilities, e.g. Incubation, Dispensing. BioTek recommends installing the latest version of basecode for your reader, which is generally free-of-charge and easy to obtain and install. Contact BioTek.

Com Port

Enter the serial communications port number. When using a **USB** connection to the PC, the Windows® operating system sets up a "virtual" Com Port. Follow the instructions below:

All BioTek Readers except the Clarity

1. Plug in the USB cable
2. For first time set up, Windows recognizes New Hardware, and prompts you to install the required driver. Follow the installation directions provided with the driver. When the wizard is finished installing the driver, a new "COM port" will be available.

3. Go to Control Panel>Administrative Tools>Computer Management>Device Manager>Ports(COM & LPT) to see the new virtual Com port assigned to the USB.
4. Enter this port for the reader.

Clarity Luminometers

1. Connect the Clarity to the PC with the USB cable.
2. Launch the Clarity software, and go to **Options>Com Port Settings**. Select USB.

Once you get this set up initially, you won't have to do it again unless you switch to a serial port connection.

3. Close the Clarity software and launch Gen5.

Gen5 will automatically use whatever connection was specified in the Clarity software. All connection information is controlled by the Clarity software only.

Baud Rate

Make sure the Baud Rate matches the reader's settings. Consult your reader's operator's manual for the correct rate. Readers without keypads, Synergy and PowerWaveXS, will issue a "serial read" error when it is powered down and then up again while Gen5 is running if the baud rate is set to other than the default setting.

Test Communication

Use this button when adding or modifying a reader to test the Com Port setting. Gen5 attempts to communicate with the reader and reports its results in an on-screen message.

Setup

Generally, the **Setup** option is **not** needed. Gen5 communicates with the reader to obtain the information it contains. Occasionally, you may want to view or modify:

- [Absorbance Wavelengths](#) (next page)
- [Fluorescence/Luminescence Filters](#) (on page 406)

Absorbance Wavelengths

System > Reader Configuration > Add/Modify button > Setup button

Use the Absorbance Wavelength tab to ensure that Gen5's wavelengths table is aligned with the reader's internal table. Depending on the reader, you can specify up to six wavelengths to be made available as default selections in the **Read Step** dialog (Protocol > Procedure > Read).

- **Get Wavelengths:** retrieve wavelength values from the instrument
- **Send Wavelengths:** download and calibrate wavelength values. Enter the desired values in the Wavelength fields and then click **Send Wavelengths**. The values will be downloaded to the instrument, overwriting its existing wavelength table.

❖ **Note:** To exchange wavelength information between Gen5 and the reader, the two must be communicating, i.e. the reader must be turned on and correctly [configured](#) in Gen5.

Reader-Specific Information

Reader Series	Wavelength range	Wavelength selection
ELx-Series	340-900 <i>depends on specific model</i>	Filters: Update the reader when changing filters
μQuant and PowerWave series	200-999 <i>depends on specific model</i>	Monochromator: selectable by 1nm increments
Synergy HT and Synergy 2	200-999	Monochromator: selectable by 1nm increments

❖ **Note:** You must conform to the specific BioTek reader procedures when altering the reader's configuration. For filter-based readers, it is your responsibility to ensure that the filters are positioned correctly and recorded here accurately.

ELx-Series Filter-Based Readers

BioTek updates the on-board software with the current configuration of filters before shipping the reader to you. Unless you change the filters without updating the on-board software, Gen5 will capture the filter-wheel configuration when it initiates communication with the reader.

If you do change the filter-wheel configuration, you can use Gen5 to update the reader:

1. Precisely record the wavelength and position of the filters before reinstalling the filter wheel
2. In Gen5, select **System>Reader Configuration** and click the **View/Modify** button
3. Click the **Setup** button, and select the **Absorbance** tab
4. Fill in the Wavelength table to match the filter-wheel configuration
5. Click **Send Wavelengths**.

Fluorescence/Luminescence Filters

System > Reader Configuration > View/Modify button > Setup button

Normally, these controls are only needed when you are **changing a filter wheel**

❖ The **Filter Wheel Library** can also update the reader's Fluorescence/Luminescence Filters table. See the Filters and Mirrors section beginning on page 165.

To change the current settings and download them to the instrument:

1. For each filter wheel, Excitation and Emission, enter wavelength values in the Center fields, or use the drop-down list to select [PLUG](#) or [HOLE](#).
2. For each [Center](#) wavelength value, enter its accompanying Bandwidth. This is the filter bandpass width, in nanometers, of the installed filter. For example, the Center/Bandwidth combination of 485/20 will transmit light from 475 to 495 nm (10 nm on either side of the center). The bandwidth is printed on the side of each filter. See the reader's operator's manual for details.
3. When all values have been entered, click **Send Values**. The values will be downloaded to the instrument, overwriting its existing wavelength table.

To retrieve filter wheel settings from the instrument:

- Click **Get Values**. This reports the values in the reader's on-board memory. Since the reader does not have the ability to mechanically determine the filter configuration, these values may NOT truly represent the current filter wheels.

Special filter position requirements for the Synchronized Modes:

For Dispensing Protocols and certain other procedures, Gen5 offers an option: **Close Light Shutter**, to block the light between measurements to inhibit photo bleaching:

- Single filter-set protocols: If light blocking is enabled, a blocking filter (Plug) must be placed in the excitation filter wheel in one of the two positions next to the excitation filter that is specified in the protocol.

- Dual filter-set protocols: If light blocking is enabled, two blocking filters (Plugs) must be placed next to each other in the excitation filter wheel, except for Synergy 2; it only needs one plug.

Changing Filter Wheels

❖ **Important!** It is essential to conform to the specific BioTek instrument procedures for altering the filter wheel configuration. The reader does not automatically detect which filters are installed. Before the instrument is shipped from the factory, BioTek updates the reader's internal software with the current filter wheel settings. When you make changes to a filter wheel, it is your responsibility to ensure that Gen5's wavelength table exactly matches the new contents of each filter wheel. For specific instructions on changing filters, see the reader's operator's manual. In order to obtain accurate results and prevent damage to the PMT, they must match exactly.

To exchange wavelength information between Gen5 and the instrument, the two must be communicating: the reader must be turned on and properly configured in Gen5. In Gen5, there are two ways to update the reader with filter wheel configuration:

- use the **Fluorescence/Luminescence** tab of the Reader Setup (**System>Reader Configuration>View/Modify>Setup**) to ensure the wavelengths table matches the filters installed in the attached reader
- use the **Filter Wheel Library** (**System> Filter Wheels**) to select a pre-defined wheel and send its values to the reader.

Reader Control Panel

System > Reader Control



25.0° or, click on the reader button in Gen5's toolbar to open the control panel.

If supported by the current reader, you can use this feature to view information about the attached reader, control the reader door or plate carrier, and control incubation.

The reader must be connected, turned on, and properly communicating with Gen5™ for the controls to be enabled. This means the reader must be "ready" not busy reading a plate or running a system test, for example.

- Information
- Door/Carrier
- [Pre-Heating](#) (below)
- [Dispenser](#) (page 409)
- [Tungsten Lamp](#) (page 413)

❖ Detailed information about the Filter Wheel and Mirror tabs is provided earlier in this guide.

Pre-Heating Parameters

System > Reader Control > Pre-Heating

In the control panel for readers with incubation capability:

- The **Set Temperature** Procedure defined for the Protocol offers the ability to activate Pre-Heating. This screen reflects the parameters defined in the protocol
- To use the reader as incubator in between experiments: enter the temperature in the **Requested** field and then check **On** to begin pre-heating. Most BioTek readers allow a temperature range of 20-50°C.
- The temperature will rise or fall to the **Requested** temperature, as appropriate. **Off** disables the incubation unit; the temperature will return to ambient.
- The **Actual** field reports the current temperature (Celsius) of the incubation unit.
 - The **Total Time** field reports the total time elapsed since the On box was checked. Associated with the Total Time field, you can use the **Beep after** checkbox to direct Gen5™ to "beep" continuously after the specified duration. The clock starts when the incubation unit is turned On.
 - The **Time Since Reached** field reports the total time elapsed since the incubation temperature reached the Requested temperature. Associated

with the Time Since Reached field, use the **Beep after** checkbox and field to tell Gen5 to "beep" continuously after a specified duration. The clock starts when the incubation temperature reaches the Requested temperature.

- **Temperature Reached** is displayed when the incubation temperature reaches the Requested temperature.

❖ **Note:** As the temperature of the incubation unit approaches the Requested temperature, it may take a few minutes to settle within an acceptable tolerance (+/- 0.5° C). During this settling period, the **Temperature Reached** indication may appear for a few seconds, disappear, then reappear moments later. The Time Since Reached field will automatically reset to 00:00:00 each time the indication appears. If the Beep after checkbox associated with Time since reached field is enabled and set to, for example, 10 minutes, Gen5 will "beep" 10 minutes after the incubation temperature has consistently settled within +/- 0.5° C of the Requested temperature.

Dispenser Settings

System > Reader Configuration > Add/Modify button > Setup button

- ❖ **Read Only:** for everyone except BioTek qualified technicians, these controls provide information, they do not let you alter it.

 Use the **Reader Control Panel** to position the dispensers for performing maintenance.

Calibration Volumes

Gen5 shows the minor differences between the expected (Target) dispensing volumes and the actual (Measured) volumes, as determined at the BioTek factory.

- Click **Get Values**. Actual measured values will be uploaded from the reader's current internal tables.

Injector Position

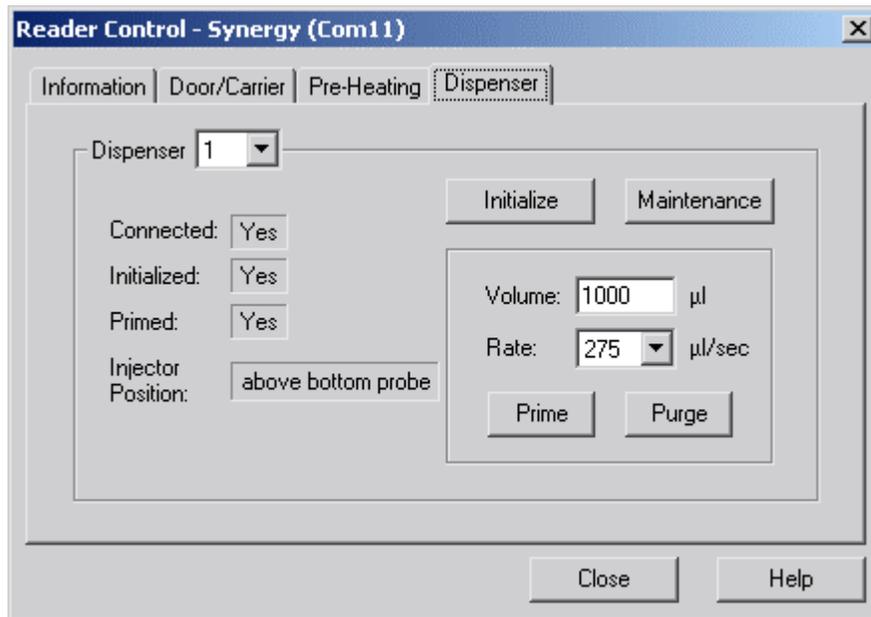
Gen5 shows the current position of the injector, if applicable to the reader.

- Next to Top Probe
- Above Bottom Probe

Also see: [Special filter position requirements for the Synergy HTTR w/Injectors](#) on page 406.

Dispenser Control and Maintenance

System > Reader Control > Dispenser



In the control panel for readers with dispensing or injection capability:

- ▼ **Dispenser:** up to two dispensers may be attached to the reader, use the drop-down list to select the one you want to control or review information about.

Dispenser Information

The fields on the left side of the screen display the current state of the selected dispenser:

- **Connected:** the reader detects a live connection (Yes) via serial cables with the dispenser module. "No" indicates a faulty connection. Check the cabling.
- **Initialized:** Prior to performing a dispense step the dispenser module must be initialized. Click the **Initialize** button if this status reports "No."
- **Primed:** Prior to performing a dispense step the dispenser must be primed. Place the priming plate on the carrier and click the **Prime** button if this status reports "No."
- **Injector position:** Gen5 reports the position of the injector in relation to the reader's probe

Dispenser Routines

- Initialize** Ensure normal and correct communication between the dispenser module and reader
- Prime** Prime the tubing with fluid

Purge

Remove and recover fluid from the tubing

Maintenance

Move the dispenser's syringe pump into maintenance position for installation or replacement

-
- ❖ When defining a Dispense Step in the Procedure you can also define **Tip Priming**
-

Priming the Dispenser

This routine is only applicable for **Readers with Injectors**. Refer to your reader's Operator's Manual for specific and more detailed maintenance guidelines.

Priming the tubing with reagent (dispensing fluid) is an important first step when running an experiment. Likewise, flushing the tubing of reagent after each use is an important maintenance step. The priming routine is used for both steps.

-
- ❖ When dispensing volumes less than or equal to 20 µl/well, we recommend specifying a tip prime volume that is equal to the dispense volume. For dispense volumes greater than 20 µl/well, we recommend a tip prime volume of 20 µl.
-

To prime the dispenser:

1. Fill the supply bottle(s) with the dispensing fluid when running an experiment or with deionized or distilled water when performing maintenance. Insert the supply (inlet) tubes into the bottles.
2. Place the priming plate on the carrier.
3. Select System > Reader Control > Dispenser
4. Set **Dispenser** to 1
5. Set the **Volume** to at least:
 - 1000 µl for pre-experiment priming
 - 5000 µl for maintenance
6. Set the dispense **Rate** (BioTek recommends 275 for priming)
7. Click **Prime** to start the process.
When the process is complete, carefully remove the priming plate from the carrier and empty it.
8. Repeat the process for **Dispenser 2**, if applicable.

 **Maintaining the Tubing:** Leave **DI** water in the system overnight or until the instrument will be used again. **Purge the fluid** from the system and then prime with the dispense reagent before running an assay.

Purging the Dispenser

This routine is only applicable for **Readers with Injectors**. Refer to your reader's Operator's Manual for specific and more detailed maintenance guidelines.

Purging the dispenser is an important part of the recommended daily maintenance routine. Gen5's purging capability can also be used to recover and preserve expensive reagents.

To purge the dispenser of fluid:

1. Remove the inlet tubes from the supply bottles.
2. Select **System > Reader Control > Dispenser**
3. Select the **Dispenser** number: 1 or 2
4. Set the **Volume** (2000µl guarantees all fluid in the system's tubing is removed).
5. Click **Purge** to start the process.
6. When the purge is complete, repeat the process for the other Dispenser, if applicable.

❖ After purging the system, you may wish to run a quick **Dispense-Only experiment** to visually verify the dispense accuracy.

Dispenser Prime/Purge Rate

The allowable volume ranges for each dispense rate are:

Rate (ul/sec)	Volume Range (ul)
225	5-5000
250	15-5000
275	25-5000
300	30-5000

BioTek recommends using the default rate of 275 for priming.

Tungsten Lamp Control

System > Reader Control > Tungsten Lamp

Turn Lamp Off

Use this control to turn off the **Tungsten Lamp** when it is not needed. Follow the menu path shown above to access the controls. Note: when the tungsten lamp is not required in a Procedure, Gen5 turns off the lamp.

Conversely, you can use the **Turn Lamp On** button. The lamp takes approximately 180 seconds to warm up before measurements can be taken. When the lamp is not warmed up before a reading is requested Gen5 displays a message that counts down this warm up period before prompting you to put the plate on the carrier.

Testing the Reader

Gen5™ provides the following options for testing the reader:

- Run a System Test
- If you've purchased BioTek's **Gen5 Reader Diagnostics Utility**:
 - [Run the Absorbance Test Plate](#)

Reader System Test

System > Diagnostics > Run System Test

❖ The System Test for the **Clarity™ Microplate Luminometer** must be performed using the Clarity PC software. Refer to the Clarity Operator's Manual for instructions.

Run the Test

Most BioTek readers perform a self test every time they're turned on, but when you want to view and/or print the results of a system (aka optics) test:

1. Select **System > Diagnostics > Run System Test**
2. When there is more than one reader attached to the PC, select the desired reader and click **OK**
3. When the test is completed:
 5. Fill in the text fields, **User, Company, Comments**, to be included in the report of the test results. Then, click **OK**.
 6. **Print** the report to retain a hard copy for your records
 7. **Save As** to convert the results to a text file. This is especially useful when troubleshooting a reader. You can email the text file to BioTek TAC.

Test History

Gen5 keeps the results of System Tests when they are performed using the menu controls. To review or print them, select **System > Diagnostics > History...**

System Test Results

System > Diagnostics > History

Immediately after running a system test on the reader, Gen5 displays the results, and then stores them in History (in the shared database).

Print

Click **Print** to generate a paper version of the results.

Save As

Click **Save As** to convert the results to a text file. This is especially useful when troubleshooting a reader. You can email the text file to BioTek TAC.

- Gen5 opens the standard Windows® file save dialog, and sets the file type to **.txt**. Text files are the default format for Notepad® and is recognized by most word processing programs.
- Optionally, click in the **File Name** field to modify the default name: SystemTest. Add the date, instrument name, or other information to ensure the file has a distinguishable name.
- Use the drop-down list and other tools to navigate to the **Save In** location

Reader Test History

System > Diagnostics > History

Gen5™ keeps this database of test results from System (formerly-called Optics) Tests and Diagnostic Tests, if applicable. It is stored in Gen5's [shared database](#).

- Double-click the desired test to open it on screen and to print it or save it as a text file

Use the options in the Test History dialog to:

-  Click the **Refresh** button to capture any tests that were conducted since the dialog has been opened
-  Use the drop-down lists in the **Selection** area to filter the list of tests shown by reader (Device) and user (Operator), if applicable
- **Column Headers** can be used to sort the tests: click on a header to sort the files in ascending/descending order by that category. For example, click Status to sort the files by Pass/Fail. Click the same column header to reverse the order
- Highlight a test and click:
 - **View:** to open it for viewing, printing, saving as a text file
 - **Delete:** to delete it, erase it from the database.

Chapter 20

System Management

This chapter supplements the Getting Started Guide shipped with the Gen5 product CD, which you should consult first for installation and initial set up instructions.

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System Requirements

Before installing Gen5™ make sure your hardware meets the minimum requirements.

Computer Requirements

For Gen5 to run successfully, the computer must meet the following requirements:

- Windows XP or Windows 2000 (Professional Editions)
- Pentium III-Class PC (or compatible) processor (500 MHz or higher)
- 512 MB RAM or higher
- 2 GB Hard Drive space or higher
- Designed for XGA Resolution at 1024 x 768 or higher
- CD-ROM Readable Drive
- Keyboard & Mouse
- Microsoft Internet Explorer v 5.0 or higher (for online Help)
- Serial or USB port for BioTek instrument

Reader Requirements

Verify that the Base Code and Assay Code built into your BioTek reader is compatible with Gen5. BioTek has validated the following list of base codes. Any instrument with the base codes listed below or higher is compatible with Gen5.

❖ If your instrument has a base code with a version lower than these please contact TAC to download and install updated software

Instrument	Base Code
Powerwave	1.21.1
PowerwaveXS	1.06
Synergy 2	1.3
Synergy HT	2.24
FLx800	1.15
ELx800	3.07
ELx808	3.15
µQuant	2.02
Clarity	NA, but version 4.0.1.10 or higher of Clarity software is required

Gen5's System Administrator

For all levels of Gen5 except Gen5 Secure

It is necessary to login as the System Administrator to change User Permissions and Database Configuration, and to access any features which are denied as **Users Permissions**

How to change the System Administrator's password:

❖ This function is only available to the **System Administrator**. You must login, **System> Administrator LogIn**, as the Administrator to access these controls

1. Select **System> User Setup**, and select the Administrator tab
2. Enter the current password in the **Current Password** field. Gen5 ships with the password set to "admin."
3. Enter the new password in both the **New** and **Confirm** password fields
4. Click **OK**.

How to maintain Users Permissions:

Except in Gen5 Secure, access to Gen5's functions, like reading a plate, modifying a protocol, and masking values, is defined equally for all users except the System Administrator, who has all "permissions."

Changing Your Password

❖ For Gen5™ Secure Only

System > Security > Users

Users other than the System Administrator are limited to changing their own login password.

How to change your password:

1. Select **System > Security > Users**
2. Identify and open your user account: highlight and click **Edit** (or double click)
3. Enter your current password in the **Current Password** field
4. Enter your new password in both the **New** and **Confirm** password fields
5. Click **OK**.

The password will take effect the next time you log into Gen5.

❖ Contact your System Administrator if you've forgotten your password. He/she can change your password without knowing the current one.

Changing Your Startup Preferences

❖ For all levels of Gen5 except Gen5 Secure

System > User Setup

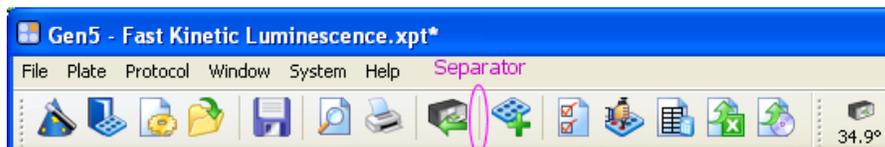
How to change your startup preferences:

1. Select **System > User Setup**
2.  Use the drop-down to select the preferred method for **Startup Action**:
 - **Display Welcome dialog** is the default setting, it opens Gen5 with a screen that offers several common tasks including creating a new item or opening a recently used item
 - **Create new experiment** opens Gen5 with the Protocol selection dialog open, as if the user had selected File>New Experiment
 - **Start at system menu** opens Gen5 showing the File, System and Help menus only. Since neither a protocol nor experiment is open, the workspace is blank.
3.  Use the **3-dot** button to change your **Protocol and Experiment Folders**: browse to the full path and directory to define the folder where you will typically store protocol and experiment files. Gen5 will point to these folders when you save and open a protocol or experiment.
4. Click **OK**.

The changes will take effect the next time you log into Gen5.

❖ Contact your System Administrator if you need assistance.

Customize the Toolbar



Double click the toolbar, anywhere without a button, to open the Customize Toolbar tool or select **System> Preferences> Customize Toolbar**

- Gen5 has two toolbars, **Protocol** and **Experiment**. Set the current state to correspond to the toolbar you want to customize: select either **File>New Protocol** or **File>New Experiment**, accordingly
- You can remove unused buttons. Add favorite buttons. Insert separators between buttons to make them easier to distinguish.
- The Customize Toolbar dialog opens with the Current Toolbar Buttons displayed in the box on the right and any unused buttons and the separator in the box on the left. The Separator is always available and there is no limitation on its use.

To remove buttons from the toolbar:

1. Highlight the button you want to remove from the toolbar in the **Current toolbar buttons** box on the right
2. Click **Remove**
The button is moved into the Available toolbar buttons box and removed from the toolbar when you click **Close**.

To add buttons to the toolbar:

1. Highlight the button you want to add to the toolbar in the **Available toolbar buttons** box on the left
2. In the **Current toolbar buttons** box, highlight the button or Separator before which you want to place the button
3. Click **Add**
The button is moved into the Current toolbar buttons box and added to the toolbar when you click **Close**.

Reset

Use the **Reset** button to restore the toolbar to its default configuration.

 To rearrange the buttons on the toolbar, first remove them and then add them in the desired location

❖ The toolbar configuration corresponds to the user logged into Windows® at the time it is customized.

Plate Types Database

System > Plate Types

About the Plate Types Database

All of the default and custom plate types currently stored in the database are listed. When a reading is initiated, Gen5 sends the appropriate plate-type details to the reader. The reader uses this information to precisely position the plate when taking measurements. Most readers support only the **Default Plate Types**: click the button to view this list. Consult your reader's operator's manual for a list of supported plate sizes.

Gen5™ comes with details for more than forty industry-standard microplates. If your reader supports custom plates, you can add your own microplates by selecting **Add**, or **Import** them from a previous version of Gen5 or KC4.

Plate Types Database Tools:

-  Click the **Default Plate Types** button to view the list and dimension details of the plate types supported by all BioTek readers
- Double click a plate type in the Default or Custom lists to review its dimensions, such as width, length, height, and the number of rows and columns. Or highlight the plate and click **View**

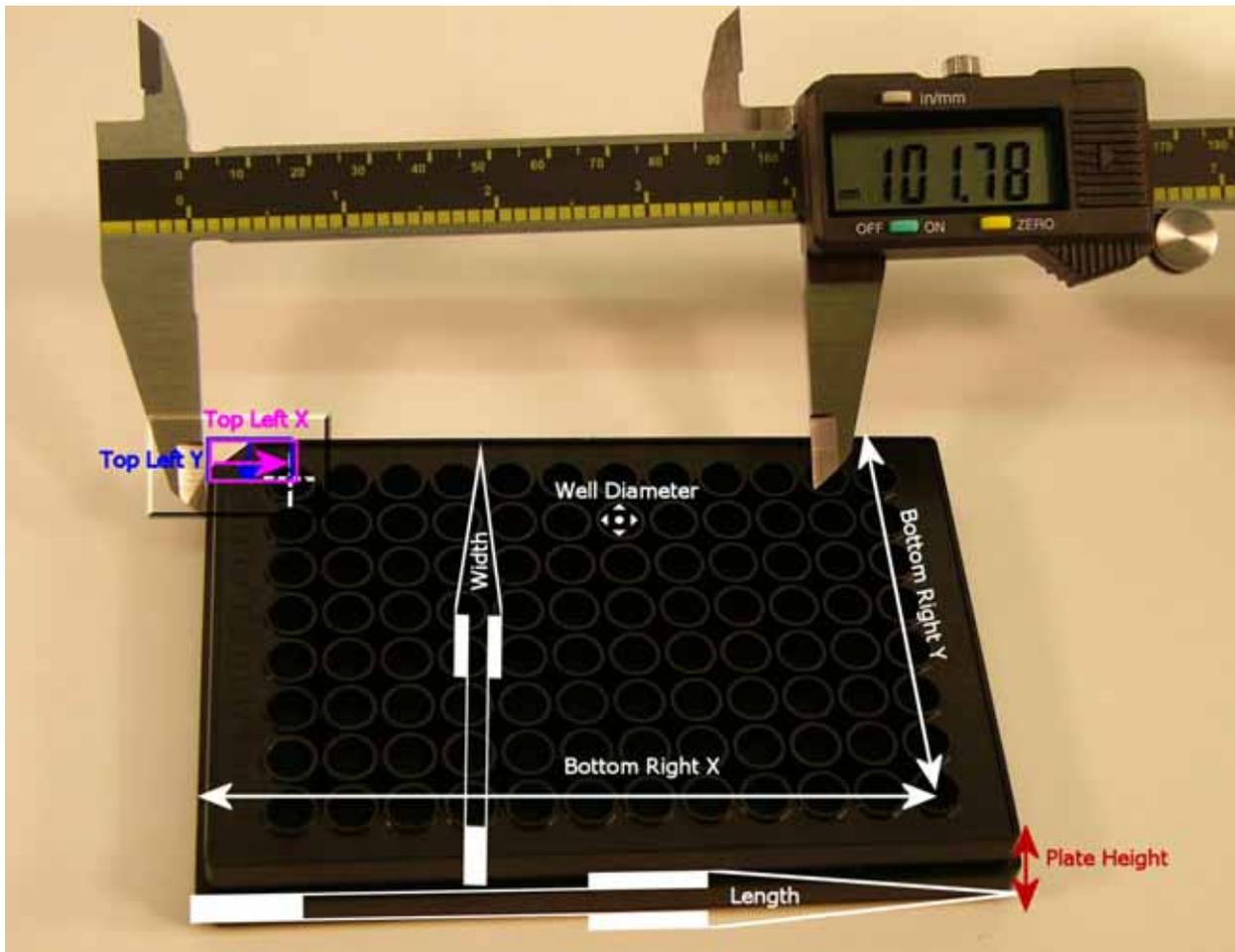
❖ **Important:** Only BioTek's Synergy and PowerWaveXS readers support Custom Plate Types.

- **Add:** to add a new plate type to the database. Take and record careful measurements of its size before creating the new record.
- **View/Modify:** to change or update the details of a plate.

❖ **Warning!** Modifying the dimensions of the installed plate types is not recommended; consider adding a new plate type instead. Please contact BioTek with any questions regarding the current dimensions.

- **Export** and **Import:** to transfer custom plate types from KC4 or to and from another Gen5 system. **Learn more...** Since the Plate Type Database is stored in Gen5's SharedDB:
 - you do not need to use the Export-Import tools to archive or backup the database
 - when the SharedDB resides on a network, all users connected to the SharedDB reference the same Plate Types Database, so you do not need to transfer custom plate types between system users
- Click **Delete** to remove a selected plate type from the database.

Plate Type Measurements



When creating or updating plate dimensions, taking precise measurements is essential. Use calipers with precision to 0.01 millimeters (mm) to gather the following measurements. A standard ruler is not precise enough. Note: when entering values based in 10 micron increments, values always end in 0 (zero).

❖ **Important!** Dimension values are used by the software to calculate reading positions. Any inaccuracies in these dimensions could significantly affect your results.

Dimensions

- **Number of Columns** is the number of vertical columns of wells when viewing the plate in its normal orientation. There are 12 in a 96-well plate.
- **Number of Rows** is the number of horizontal rows of wells when viewing the plate in its normal orientation. There are 8 in a 96-well plate.
- **Length** is the longest dimension of the plasticware (the x axis).
- **Width** is the shorter dimension of the plasticware (the y axis).

- **Height** is the distance from the bottom mounting surface of the plate to the top face of the plate.
- **Well Diameter** is the diameter of any well.
- **Top Left X** is the distance from the left side of the plate to the center of well A1.
- **Top Left Y** is the distance from the top of the plate to the center of well A1.
- **Bottom Right X** is the distance from the left side of the plate to the center of the last well on the plate. In a 96-well plate, this is H12.
- **Bottom Right Y** is the distance from the top of the plate to the center of the last well on the plate. In a 96-well plate, this is H12.

Import and Export Plate Types

System > Plate Types

Gen5 provides the Export and Import feature to transfer custom plate types from KC4 or to and from another Gen5 system. It may be necessary, for example, to import a custom plate type associated with a Gen5 protocol you've received from BioTek or another Gen5 system user.

The Import routine examines the plates in the import file and compares them to the current plate type files. New plate types are added to the database. Existing plate types with new dimensions will replace the existing dimensions after user confirmation. Existing plates with identical dimensions are ignored. Gen5 Secure logs the event in the System Audit Trail.

How to Import Plate Types

When you have one or more custom plate types exported from KC4 or Gen5 (and therefore in the proper file format):

1. Click **Import**. Gen5 opens the standard Windows® browse dialog.
2. Locate the plate type file and click **Open**.
Gen5, by default, looks for files with the **.ptf** extension, but, it will accept a file with any extension, as long as the data is correctly formatted. Change the **Files of type**: using the drop-down list to see all file types.

How to Export Plate Types

When you want to export one or more custom plate types:

1. Highlight the files in the **Custom Plate Types** box. Hold the **Ctrl** key to select multiple files.
2. Click **Export**. Gen5 opens the standard Windows® Save As dialog.
3. Browse to the storage location for the files, enter a **File name** and click **Save**.
Gen5, by default, assigns the **.ptf** extension to the file, but you can change it, if desired, by typing a different filename extension.

Chapter 21

Troubleshooting

This chapter is intended to help you resolve or recover from error messages or other system trouble. The Fluorescence and Luminescence chapter also contains troubleshooting suggestions particular to those detection methods.

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Troubleshooting

Here are some guidelines for error recovery, find additional information in Gen5's Help:

- **First Response: [Running a System Test](#)** is the best first response to a reader error. The test may restore the reader's initial settings and computer communication capability. Note: to stop the alarm on readers without keypads (e.g. Synergy) press the plate-carrier button
- **Reboot your Computer and Reader:** When you can't run a system test, e.g. Gen5 is not responding, or when running a system test doesn't resolve the issue, turn off your computer and reader, check all the cabling, i.e. make sure your serial or USB cable is in good condition and is properly connected to the PC and reader, and then, power on your computer and reader. This should refresh the devices and reset communication parameters
- **[Communication Errors: PC to Reader](#)** on page 427
- **[Database Error Recovery](#)** in the **Managing Files** chapter
- **[Calculation \(Data Reduction\) Warnings](#)** on page 429
- **[Reader Error:####](#)** on page 428
- **[Handling Other types of Error Messages](#)** refer to Gen5's Help
- **[Unknown System Admin's Password](#)** on page 432
- **[Computer Performance Slowdown](#)** on page 432
- **[Troubleshooting Fluorescence/Luminescence Measurements](#)** in a previous chapter on page 161
- Excel® errors refer to Gen5's Help

 Visit BioTek's website for useful suggestions on getting the most from your reader: <http://www.biotek.com/products/technotes.php>.

Communication Errors: Reader to Computer

Important Information:

- To prevent damage to the reader, always turn OFF the reader or the computer before removing or inserting a communications (serial or USB) cable
- Gen5™ and the reader-communication parameters will supersede the Windows® settings. Windows communication port configuration settings should **not** need adjustment to enable proper communications

When the computer (PC) won't communicate with the reader:

1. **Confirm that the reader passes its system self test.** Most readers perform a self test when turned on. Refer to the reader's user guide for more details. The reader will not communicate if it fails an internal system test. If the reader fails, refer to the user's guide to resolve the failure.
2. **Make sure the serial or USB cable is in perfect condition and properly attached** to the port defined in the Reader Configuration dialog (e.g. COM 1). Correct and reboot both PC and Reader. [Test communication](#).
3. **Confirm the Baud rate** (or transmission speed) defined in Gen5's [Reader Configuration](#) matches the reader's settings. Consult your reader's user guide for the correct rate. Correct Gen5's Reader Settings to match the reader and reboot both PC and Reader. Test communication.
4. **Disable the Calculation Option: Perform data reduction after each read** to give Gen5 sufficient time between obtaining measurements to perform calculations
5. **Confirm that the serial cable was obtained from BioTek.** Serial cables are not universal. Consult the reader's user guide for proper cable configuration or contact BioTek customer service to purchase a factory tested cable. After installing a known, good cable, reboot both PC and Reader. Test communication.
6. **Confirm** with your computer supplier or a local PC technician that **the serial port has been enabled**. For example, the IBM Thinkpad® was originally shipped with the serial port disabled. Correct and reboot both PC and Reader.
7. **For advanced PC users**, the serial port of the reader and PC can be independently tested using an independent serial-communication software package such as Windows Terminal™, Hyper Terminal™, or ProCom™. BioTek does not support or sell these programs.
 - Select flow control for "XON/XOFF" and send an ASCII asterisk symbol (*) to the reader. The reader should initiate a self test and return the results to the PC. If the reader fails to communicate, test the reader on an alternative PC to confirm which device is at fault. Please contact BioTek if the reader is diagnosed to be faulty.

Error Messages

Here are some guidelines to help you quickly resolve an error:

1. Make note or take a "[print screen](#)" of the error message
2. Locate and follow the specific instructions/suggestions for the error provided in the reader's operator's manual
3. Call BioTek's Technical Assistance Center (TAC) if you are unable to resolve the issue yourself.

Potential Error Messages and their Resolutions

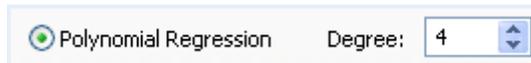
- **Reader Error ###:** refer to the reader's Operator's Manual to identify the specific error. You may be able to resolve it yourself.
- **File Not Found:** there are several potential causes for this error. In a shared database environment, another user may have the file open. Check with your colleagues to eliminate this as the cause. When using the Windows® File System for file storage, use Windows Explorer to verify the location of the file.
- **Bandpasses overlap:** the selected Excitation and Emission filters are too close. Change one of the selected filters. Learn more in the Fluorescence and Luminescence chapter.
- **Baud rate settings can cause "serial read" errors:** when the baud rate is set to a non-default setting for Synergy and PowerWaveXS readers, Gen5 may be unable to communicate with them if they are turned off and then turned on again while Gen5 is running. Gen5 may reset the baud rate to the default setting in this scenario. [Run a System Test](#) to return readers to their initialized state

Calculation Warnings

When Data Reduction steps generate an error or cannot be calculated, Gen5 displays and logs a Calculation Warning. Here are some guidelines for pinpointing and correcting the source of the error.

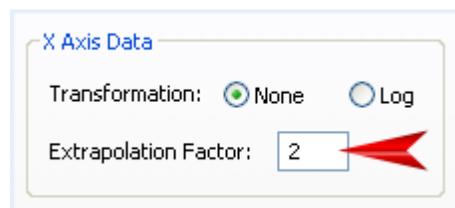
Curve Fit

- [Not enough data points to fit](#)
If the plate was read successfully, i.e. all the data points obtained, make sure you have defined the minimum number of X-Axis points, e.g standards, for the selected curve fit. See [Curve Fit: Minimum Number of Standards](#) in the Data Reduction chapter.
- [Too many data points](#)
The Spline curve fit is the only option with a limit on the number of X-axis points. Either change the curve fit method or reduce the number of standards, dilution samples, or calibrators, i.e. the number of X-data points.
- [The selected curve fit method does not accept data points with identical X values](#)
The Spline and 4-P curve fits cannot be plotted when X-axis values are identical. Either:
 - select a different [curve fit](#)
 - change the [Data In](#) selected for the X-axis
 - if the selected X-axis data is based on a Transformation, rewrite the formula to suppress duplicate values in the results
- [The degree of the polynomial regression fit has been reduced](#)
If desired, you can either:
 - Reduce the Degree
 - Add more X-axis points, e.g. standards



- [Math overflow error](#)

Check the **Extrapolation** setting



or **Interpolation** formulas (on the Data Out tab) to make sure they are not raising values to too high a value.

- [Curve fit generic error](#)

Is the curve fit 4-P?

Formula: $Y = (A-D)/(1+(X/C)^B) + D$

If so, this error may be indicating that the calculated B parameter ≤ 0

- [Curves do not support multi-indexed data sets](#)

It is possible the Procedure was changed from an endpoint read to a kinetic analysis (or other multi-index option) without revising the curve generator.

Review the Data Reduction steps and make the necessary adjustments, e.g. Well Analysis may be a better fit.

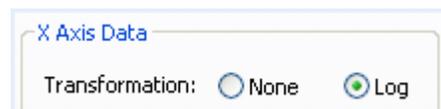
- [The 4-parameter fit is not convergent. It is probably not the best fit](#)

Gen5 performs multiple approximations to plot a 4-P curve. It is expected that the err (error) will decrease with each approximation, indicating a better fit is being determined during each iteration. Gen5 displays this message when the err does not decrease.

Select another curve fit method.

- [Error during calculation. Data to fit not compatible with axis type](#)

Review the **Data Reduction > Curve Analysis** definition, is the Axis Data set to Log? and some of the **Data In** values are < 0 ?



If yes, change these settings to eliminate the error.

Validation

- [At least one validation condition failed](#)

This message alerts you to the results of a **Data Reduction > Validation** formula. A criterion of your experiment may not have been satisfied. Review the results.

Cutoff

- [Cutoffs do not support multi-indexed data sets](#)

It is possible the Procedure was changed from an endpoint read to a kinetic analysis (or other multi-index option) without revising a **Data Reduction > Cutoff** step.

Review the Data Reduction steps and make the necessary adjustments, e.g. it may be possible to use a Validation formula in place of Cutoff results.

- [Cutoffs are not in increasing order. Symbols can not be determined](#)
Review the **Data Reduction > Cutoff** formulas. They must be input in ascending order, from the lowest (obtained or calculated) value to the highest.
- [Some cutoffs could not be calculated. Symbols cannot be determined](#)
Review the **Data Reduction > Cutoff** formulas. Try rewriting them, making sure to reference valid Well IDs, non-masked data values and logical formulae.

Well Analysis

- [Well Analysis requires multi-indexed data set](#)
It is possible the Procedure was changed from a kinetic analysis (or other multi-index option) to an endpoint read without revising the Well Analysis step.
Review the Data Reduction steps and make the necessary adjustments or update the Procedure to define kinetic or scanning analysis to generate a multi-index data set.

Restoring Optimal Performance

Numerous factors can affect your computer's performance. If you notice a slowdown in Gen5's performance, follow these suggestions:

- **Close all other applications**, including Internet browsers, when running Gen5
- **Do not display Gen5's "Curves" data** in the **Plate View** while performing a kinetic analysis. Wait until the read step is finished before viewing the "Curves" data set. Displaying the **Curves** data set during a Kinetic read can consume excessive resources resulting in performance degradation. You can drill down to a **Well Zoom** to monitor the progress of one well, then, leaving the Well Zoom open, change the Matrix Data to a numeric view
- **Disable the Calculation Option "Perform data reduction after each read"** to give Gen5 sufficient time between obtaining measurements to perform calculations
- **Disable the auto-Save options for interim reads:** Save Options can be set to free up resources.

System Administrator's Password

Contact BioTek Customer Care if you've lost or forgotten the System Administrator's password: **BioTek Customer Care**

The System Administrator's password does not expire, but if a change in personnel, or some other cause has resulted in your team not knowing the password, you can contact BioTek for a new one.

Gen5 ships with the System Administrator's password set to "**admin.**"

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