Sample Preparation for FACS Sorting

Working from a single-cell (no aggregates) suspension is crucial for any Flowcytometric Experiment. (Unless clusters are the focus of the experiment)
Cells, which are taken from a natural suspension (e.g. blood), require less effort in preparation for Flowcytometry than adherent cells or solid tissue.
Please review below information on suggestions how to prepare either type of cells.

Preparation Method for Suspension Cells

1. Transfer cells directly from the culture flask into 50-mL conical tubes and centrifuge at 1200 rpm for 5 min.
2. Discard the supernatant and re-suspend the cells in media (FACS buffer).
3. Centrifuge at 1200 rpm and discard supernatant. Re-suspend the cells aiming at a concentration of about 1×10^7 cells per mL. Be aware that the previous preparation steps will have caused loss in cells. If possible count the cells before re-suspension.
In case the detrimental effects (e.g. clumping) are apparent, the sample may be diluted or 5 mM EDTA added.
4. Immediately before sorting, filter the suspension through a 40 micron mesh.

Preparation Method for Adherent Cells

1. Harvest cells by using trypsin. Transfer cells to 50-mL conical tubes and centrifuge at 1200 rpm for 5 min.
2. Discard the supernatant and re-suspend the cells in media (FACS buffer).
3. Centrifuge again at 1200 rpm and discard supernatant. Re-suspend the cells in a small volume of FACS buffer and repeatedly re-suspend the cells using a pipette in order to break up clumps. Re-suspend the cells with a maximum concentration of 1×10^7 cells per mL. Be aware that the previous preparation steps will have caused loss in cells. If possible count the cells before re-suspension.
In case the detrimental effects (e.g. clumping) are apparent, the sample may be diluted or 5 mM EDTA added.
4. Immediately before sorting, filter the suspension through a 40 micron mesh.

Preparation from Solid Tissue

1. Place tissue in a sterile Petri dish. Tease tissue apart using needle and scalpel. In addition, enzymatic disaggregation (e.g., collagenase (220 U/mL) may also help free single cells.
2. Decant cells into a 50-mL conical tube and centrifuge at 1200 rpm for 5 min.
3. Discard the supernatant and re-suspend in FACS buffer.
4. Centrifuge again at 1200 rpm and discard supernatant. Resuspend the cells in a small volume of FACS buffer and count cells as above. Be aware that the previous preparation steps will have caused loss in cells. If possible count the cells before re-suspension.
In case the detrimental effects (e.g. clumping) are apparent, the sample may be diluted.
5. Immediately before sorting, filter the suspension through a 40 micron mesh.

FACS buffer

1x PBS with 5% FBS (and optional 0.1% NaN3 sodium azide). Do not add sodium azide to buffers if you are concerned with recovering cell function e.g. if cells are to be collected for functional assays. It inhibits metabolic activity.