Mass Spectrometry

Gel Digestion Protocol

Gel digest - Trypsin

1. **Cut the gel:**
   - Cut out bands you prefer and than cut each band in 2-3 mm pieces, put them in a 0.6 ml PCR tube from AXYGEN.

2. **Wash the gel pieces:**
   - Use the shaker for each wash step at RT for 10 min. Remove the supernatant after each step.

   - 200 µl 100 mM ABC.
   - 100 µl ACN, 100 µl 100 mM ABC.
   - Repeat the two steps above.

   - Remove the supernatant and add 100 µl ACN to shrink the gel-pieces.

   Note: if the bands are heavily coomassie-stained, you might wash them over night in 100 µL ACN, 100 µL ABC 100 mM on the shaker at RT.

3. **Reduction:**
   - Cover the gel-pieces with 100 µl of 1 mg/ml DTT in 100 mM ABC.
   - Incubate at 57°C for 30 min.
   - Remove supernatant.

4. **Alkylation:**
   - Cover the gel-pieces with 100 µl of 28 mM MMTS in 100 mM ABC.
   - Incubate at RT in the dark for 30 min.
   - Remove supernatant.

5. **Wash the gel pieces:**
    - Perform the same wash steps which you did in step (2). Remove supernatant and shrink the gel-pieces as in step (2).
    - Dry the gel pieces in the speed-vac for 5-7 min
    - Close tubes and flick them carefully – dry gel pieces are loose and move around. If the don't, put tubes back in the speed vac for a few min

Note: It is now possible to store the gel-pieces at -80°C.

6. **Trypsin digest:**

Use only “trypsin gold” from “Promega” and our stock-solution (c = 100 ng/ul). Dilute it with 100 mM ABC to c = 12 ng/µL.
This is a 1:8 dilution step:
=> 160 µL aliquot: 20 µL stock + 140 µL ABC.
=> 80 µL aliquot: 10 µL stock + 70 µL ABC.
=> 40 µL aliquot: 5 µL stock + 35 µL ABC.

- Cover the gel pieces with 20 µL diluted trypsin/ABC solution and put it at 4-5°C for 5 min. If the gel-pieces suck in all of the solution, pipette another few µL of diluted trypsin/ABC solution on them and let them rest again for 5 min.
- Remove the supernatant and add the same volume of 100 mM ABC as you used before with the trypsin solution (20 µl or more).
- Incubate overnight at 37°C.

7. **Extract gels:**
    - Pipette the supernatant (~20 µl) into a 0,2 ml PCR tube and store it at 5°C.
    - Cover the gel pieces with 20 µL 5% formic-acid and sonicate them for 10 min in a *cooled* ultrasonic bath.
    - transfer the 20 µL supernatant to the 0,2 ml PCR tube from the 1st step and put the tube in the fridge again.
    - Once more, cover the gel-pieces with 20 µL 5% formic-acid and sonicate them in a *cooled* ultrasonic bath.
    - Again, transfer the supernatant to the 0.2 ml PCR tube.
    - Discard the gel pieces.

You should now have a 0.2 ml PCR tube with ~ 60 µL of your gel digest.

Gel digest - Chymotrypsin

The steps are the same as in the “trypsin digestion protocol”, there is only one important difference in step Nr. 6:

6. Chymotrypsin digest:

- Incubate at 25°C for 5h.

Gel digest - Subtilisin

1 – 5. Prepare the gel pieces:

- Prepare (cut, wash, reduce, alkylate, wash) the gel-pieces as described in the steps 1-5 of the “trypsin digestion protocol”.

6. Subtilisin digest:

Subtilisin has to be prepared freshly:

- Prepare the Urea/Tris dilution solvent by mixing 9 vol. parts of 6 M Urea with 1 vol. part of 1M Tris (eg.: 1800 µL Urea + 200 µL Tris).
- Prepare 5 mg subtilisin in 500 µL 1mM HCl (c = 10 µg/µL).
- Dilute this 1:5 with 1mM HCl. (c = 2 µg/µL).
- Dilute this 1:160 with Urea/Tris dilution solvent (c = 12.5 ng/µL).
- Dilute this 1:2 with Urea/Tris dilution solvent (c = 6 ng/µl).

- Cover the gel-pieces with 30 µL subtilisin solution and put it at 4-5°C for 5 min. If the gel pieces suck in all of the solution, pipette another few µL of subtilisin solution on them and let them rest again for 5 min.
- Remove the supernatant and add the same volume of 100 mM ABC as you used before with the subtilisin solution (30 µl or more).
- Incubate at 37°C for 1h on the shaker.

7. Extract gels:

- Pipette the supernatant (~30µl) into a 0,2 ml PCR tube and store it at 5°C.
- Cover the gel pieces with 20 µL 5% formic acid and sonicate them for 10 min in a *cooled* ultrasonic bath.
- After sonication, transfer the 20 µL supernatant to the 0,2 ml PCR tube from the 1st step and put the tube in the fridge again.
- Once more, cover the gel-pieces with 20 µL 5% formic acid and sonicate them in a *cooled* ultrasonic bath.
- Again, transfer the supernatant to the 0.2 ml PCR tube.
- Discard the gel pieces.

You should now have a 0.2 ml PCR tube with ~ 70 µL of your gel digest.

Gel digest - LysC

The steps and amounts are the same as in the “trypsin digestion protocol”