In-gel protein digestion

Reagents

50 mM ammonium bicarbonate pH 8.5 (NH_4HCO_3, 200 mg/50 ml)

100% acetonitrile (ACN)

6.5 mM DTT (1 mg/ml in 50 mM NH_4HCO_3)

54 mM iodoacetamide (IAA, 10 mg/ml in 50 mM NH_4HCO_3)

Trypsin stock, 100 µg/ml in 50 mM acetic acid (store at -80 °C)

Silver destaining solution (if needed), 99 mg potassium ferricyanide $K_3Fe(CN)_6$ + 250 mg sodium thiosulphate $Na_2S_2O_3$ in 10 ml milliQ water

Formic acid (FA)

Procedure

Upon electrophoresis, gel can be stained using Coomassie blue for 1 hour. Destain gels overnight using destain buffer (10% methanol + 7% acetic acid). Then wash with distilled water for 2 hours or so.

1. Put the gel side on a glass pad (previously wiped with cleaning spray or methanol), wet with water, excise bands or lanes of interest, chop into small cubes (1×1 or 2×2 mm) under flow cabinet to prevent contamination, and place into an Eppendorf tube.
2. Wash the gel bands with 250 µl miliQ twice. Shake it to make sure all pieces are in solution.

If needed, destain the silver-stained gel piece with 100 µl silver destaining solution until the colour disappears, remove this and wash the gel piece with 100 µl miliQ and continue with step 3.

3. After removing water, add 250 µl ACN and shake the Eppendorf tube to shrink the gel pieces (you will see the gel pieces stick together).
4. Remove the ACN.
5. Add 6.5 mM DTT and incubate at 60 °C for 1 hour with shaking (300 rpm).
6. Remove DTT and wait for the solution to cool down to room temperature.
7. Add 250 µl ACN to shrink the gel pieces, shake or vortex and remove the ACN.
8. Add 100 µl IAA, incubate in the dark for 30 minutes (wrap the tube in aluminium foil and leave in a drawer).
9. Remove the IAA. Add 100 µl ACN and shake or vortex. Remove the ACN.
10. Add 100 µl 50mM NH4HCO3 to wash. Remove the NH4HCO3.
11. Repeat steps 9 and 10.
12. Remove the supernatant as much as possible (ACN would denature the trypsin). The gel pieces must remain shrunk.

13. Dilute stock trypsin with 50 mM NH_4HCO_3 to 3 ng/µl for 5 µg protein (depends on the amount of protein loaded onto the gel) and add approximately 30 µl to cover the gel pieces.
14. Leave on ice for 30 minutes (can be longer).
15. Remove the excess trypsin and add 30 µl 50 mM NH_4HCO_3 to incubate overnight at 37 °C with shaking (300 rpm).
16. Transfer the supernatant to a new vial.
17. Add 30 µl 50 mM NH_4HCO_3 (or 20 µl 10% FA) to the gel pieces to extract the remaining peptides in the gel. Remove this supernatant and transfer to the first one.
18. Repeat step 17.
19. Dry the supernatant down and resuspend in 0.1% FA for LC-MS analysis.