IN-GEL DIGESTION PROTOCOL

Solutions Used During Protocol

25mM Ammonium Bicarbonate
25mM Ammonium Bicarbonate / 70% ACN
10mM DTT in 25mM Ammonium Bicarbonate
50mM Iodoacetamide in 25mM Ammonium Bicarbonate
10ng/µl Trypsin in 25mM Ammonium Bicarbonate
50% ACN / 5% Formic Acid (or TFA)

Keratin contamination is a major hazard of in-gel digestion. Therefore, 'powder free' gloves should be worn at all times during the protocol, and caution should be taken at all stages to minimize risk of contamination; i.e. lids on tubes, tip boxes and solutions should be kept on as much as possible.
In order to minimize sample loss, siliconized (hydrophobic coated) tubes are used throughout the process.

Wash/Dehydrate Gel Pieces – to wash gel pieces and remove coomassie stain.

1. Add 50µl 25mM Ammonium Bicarbonate /70% ACN to gel pieces and incubate for 10 min.
2. Remove supernatant using a gel-loading pipette tip.
3. Repeat steps 1 and 2

Reduction and Alkylation – to reduce disulphide bonds and modify cysteines to prevent bonds from re-forming.

4. Add 30µl 10mM DTT in 25mM Ammonium Bicarbonate to dried gel pieces and incubate at 50°C for 45 minutes.
5. Remove supernatant using a gel-loading pipette tip. Add 30µl 50mM Iodoacetamide in 25mM Ammonium Bicarbonate and incubate in dark for 1hr.
6. Remove supernatant using a gel-loading pipette tip. Add 30µl 25mM Ammonium Bicarbonate / 70% ACN and incubate for 5 min, then repeat this step.

Digestion

7. Add enough trypsin solution to barely cover gel pieces. For a 2D spot this will be about 5µl, for a 1D band 10-15µl. Allow gel pieces to swell for 5 minutes.
8. Overlay gel pieces with a further 30µl 25mM Ammonium Bicarbonate and incubate at 37°C for 4hrs (or overnight).

Peptide Extraction

9. Remove and collect supernatant in a new siliconized tube using a gel-loading pipette tip
10. Add 30µl 50%ACN / 5% Formic Acid and incubate for 10 minutes.
11. Remove and collect supernatant using gel-loading pipette tip in same siliconized tube as step 11.
12. Speed-vac collected supernatant down to 5-10µl.
13. Add 5µl 0.1% Formic Acid.