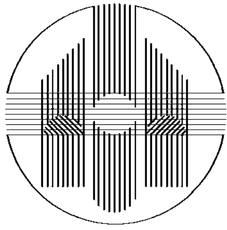

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Protocol

In-Gel Digestion by Trypsin

- Excise spots/bands cutting as close to the edge of the spot/band as possible (to reduce the volume of 'background' gel)
- Transfer gel plug into a reaction tube (Eppendorf)
- Prepare the following solutions:

25 mM NH_4HCO_3 (100 mg/50 ml)
25 mM NH_4HCO_3 in 50% ACN
10 mM DTT in 25 mM NH_4HCO_3 (1.5 mg/mL)
55 mM iodoacetamide in 25 mM NH_4 HCO_3 (10 mg/mL)
50% ACN/5% formic acid (may substitute TFA or acetic acid)
12.5 ng/µL trypsin in 25mM NH_4HCO_3 (freshly diluted)

- Add ~100µL of 25mM NH_4HCO_3/50% ACN for 10 min
- Remove the supernatant and discard.
- Repeat steps 4 and 5 once or twice.
- Add 25 µL 10 mM DTT in 25 mM NH_4HCO_3. Allow reaction to proceed at 57 °C for 1 hr.
- Remove supernatant, add 25 µl 55 mM iodoacetamide to the gel pieces. Allow reaction to proceed in the dark for 45 min at room temperature.
- Remove supernatant. Wash gels with 100 µl NH_4 HCO_3 for 10 min
- Remove supernatant. Wash gels with 100µL of 25 mM NH_4HCO_3 in 50% acetonitrile for 10 min.
- Remove supernatant. Add 100µL of acetonitrile for 10 min to shrink the gels.

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- Remove supernatant completely.
- Add trypsin solution to just barely cover the gel pieces. Estimate the gel volume and add about 3x volume of trypsin solution. This volume will vary from sample to sample, but on average 5-25 µL is sufficient.
- Incubate at 37°C for 4 hours - overnight.
- Supernatant is used for analysis by MALDI TOF MS or ESI QTOF MS.

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