Arizona Proteomics Consortium Protocol
revised May 16 2012

Trypsin digestion of protein in gel bands

based on Shevchenko et al., Anal. Chem. 68, 850-858 (1996)

Equipment

heat block or water bath
bath sonicator
speed vac
pH paper

Reagents

Sequencing grade modified trypsin (we use Princeton Separations cat #EN-151 or Promega cat# V511), 12.5 ng/µl trypsin in 50 mM Ambic.
Nanopure or equivalent H_2O
Acetonitrile (ACN)
100 mM ammonium bicarbonate, pH 8.0 (Ambic, 7.9 mg/mL ddH_2O)
200 mM DTT (30.8 mg DTT/mL ddH_2O)
10 mM DTT (50 ul 200 mM DTT + 950 ul 100 mM Ambic)
55 mM iodoacetamide (10 mg IAA/mL 100mM Ambic)
Trifluoroacetic acid (TFA) (we use Pierce cat # 28904)

For all steps use sufficient solution volumes to completely cover the gel band. For small gel bands or spots use gel loading pipette tips to avoid losing the band by aspirating it.

Washing Coomassie blue stained bands

1. Before excising bands wash gels in ddH_2O for 15 min.
2. Excise bands, cut as close to the band as possible to minimize excess gel material.
3. Chop bands into $1mm^3$ pieces.
4. Place bands in clean Eppendorf tubes.
5. Add ddH_2O to each band and incubate for 15 min.
6. Remove ddH_2O, add 50/50 ACN:ddH_2O and incubate for 15 min.
7. Remove solution and add 100% ACN, incubate until gel pieces are white and sticky (darkly stained bands may be a bit blue).
8. Remove ACN and add 100 mM Ambic, incubate 5 min.
9. Add an equal volume of ACN to make 1:1 solution, incubate 15 min.
10. Remove solution and dry samples in speed-vac for approx. 15 min. (Must be very dry!).

Washing silver stained bands

Bands should be destained the same day the gel is stained, refer to the Destaining Protocol for Silver Stained Gel Bands.

1. Add ddH_2O to each band and incubate for 15 min.
2. Remove ddH_2O, add 100 mM Ambic and incubate for 15 min.
3. Add an equal volume of ACN to make 1:1 solution, incubate 15 min.
4. Remove solution and dry samples in speed-vac for approx. 15 min. (Must be very dry!).

Reduction of disulfide bonds and alkylation of cysteines

1. Remove samples from speed-vac and let cool.
2. Add 10 mM DTT and incubate at 56°C for 45 min.
3. Remove samples and cool to RT.
4. Remove solution and immediately add 55 mM iodoacetamide and incubate at room temperature for 30 min in the dark.
5. Remove solution and wash with 100 mM Ambic, incubate 5 min.
6. Add an equal volume of ACN to make 1:1 solution, incubate 15 min.
7. Remove solution and dry gel pieces in speed-vac for 15 min or until very dry.

Digestion

1. Add freshly prepared trypsin digestion solution and incubate on ice for 45 min.
2. Remove and discard trypsin solution, add 50 mM Ambic, ensure that the gel band is well covered by the buffer, and incubate overnight at the trypsin manufacturer's recommended temperature.

Extraction of tryptic peptides

1. Acidify the digestion using 10 % TFA to bring pH to around 3 (test on pH paper), wait 1-2 minutes, remove the supernatant and save in clean microcentrifuge tube.
2. Cover the gel slice with TFA:ACN (0.1%:60%), place in a floating rack in a sonicating water bath and extract for 30 min with sonication. Add ice to the bath as necessary to maintain the temperature around 20°C. Combine supernatant with previous solution. Speed-vac pooled supernatants to remove TFA/ACN (reduce solution to approximately 10 µl), vortex, and store at -20°C until analysis.

Notes

- Minimize keratin contamination by wearing a lab coat, tying back hair if it's long, wearing powder-free gloves and changing them frequently. When excising gel bands use a methanol cleaned glass plate and scalpel or razor blade.
- Chymotrypsin digestion: 1.25 ng/µl protease, include 10 mM CaCl2 in the protease buffer.