In-Gel Digest Protocol

Stock Solution:

1. 25 mM NH_4HCO_3 (100 mg/50 ml)
2. 25 mM NH_4HCO_3 in 50% ACN
3. 50% ACN/5% formic acid (may substitute TFA or acetic acid)
4. 12.5 ng/µL trypsin in 25mM NH_4HCO_3 (freshly prepared)
5. 10 mM (1.5 mg/mL) DTT in 25 mM NH_4HCO_3 (freshly prepared)
6. 55 mM (10 mg/mL) iodoacetamide in 25 mM NH_4HCO_3 (freshly prepared)

Procedure:

1. Dice each gel slice into small pieces (1 mm^2) and place into 0.65 mL siliconized tube.
2. Add ~100µL (or enough to cover) of 25mM NH_4HCO_3/50% ACN and vortex for 10 min.
3. Using gel loading pipet tip, remove the supernatant and discard.
4. Repeat steps 3 and 4 once or twice.
5. Speed Vac the gel pieces completely dry (~ 20 min).
6. Add 25 µL (or enough to cover) 10 mM DTT in 25 mM NH_4HCO_3 to dried gels. Vortex and spin briefly. Allow reaction to proceed at 56°C for 30 min.
7. Discard the supernatant, add 25 µl 55 mM iodoacetamide to the gel pieces. Vortex and spin briefly. Allow reaction to proceed in the dark for 45 min at room temperature.
8. Discard supernatant. Wash gels with ~100 µl NH_4HCO_3, vortex 10 min, and spin.
9. Discard supernatant. Dehydrate gels with ~100µL (or enough to cover) of 25 mM NH_4HCO_3 in 50% ACN, vortex 5 min, spin. Repeat one time.
10. Dry the gel pieces completely with speed vacuum (~20 min). Proceed with trypsin digest.
11. Add trypsin solution to just barely cover the gel pieces. Estimate the gel volume and add about 3× volume of trypsin solution. This volume will vary from sample to sample, but on average ~5-25 µL is sufficient. Rehydrate the gel pieces on ice or at 4°C for 10 min. Spin.
12. Add 25mM NH_4HCO_3 as needed to cover the gel pieces.
13. Spin briefly and incubate at 37°C for 4 hours – overnight.
14. Transfer the digest solution (or extraction) into a clean 0.65 mL siliconized tube (one per sample).
15. To the gel pieces, add 30 µL (enough to cover) of 50% ACN/5% formic acid, vortex 20-30min, spin, sonicate 5 min. Repeat steps 1 and 2.
16. Combine and vortex the extracted digests, spin and Speed Vac to reduce volume to 10 µL.
17. Either proceed with C18 ZipTip (Millipore) cleanup or analyze with LC-MS. Add 2-5 µL of 5% formic acid. When analyzing low levels of protein, concentrate the peptides by eluting from ZipTips using 3µL of elution solution into a clean 0.65 mL siliconized tube.

Adapted from UCSF MS Facility: http://ms-facility.ucsf.edu/ingel.html