Large scale in-solution digests (for 2D-LC analysis)

Reagents:

1. 4X digestion buffer: 8 M electrophoresis grade urea (deinionized), 1.0 M Tris, 0.2 M methylamine, 8 mM $CaCl_2$ (pH 8.5) (freeze in 1 ml aliquots).

2. 0.9 M Dithiothreitol (DTT) solution (35 mg into 0.25 ml of water) Make up fresh.

3. 1.0 M Iodoacetamide (IAA) solution (46 mg into 0.25 ml). Make up fresh.

4. Dissolve 100 µg vial of trypsin gold (ProMega) in 100 µl of water, keep on ice.

Protocol:

1. Start with a vacuum concentrated sample containing 1-5 mg of protein that contains nothing that would inhibit the digestion (protease inhibitors, detergent, buffers with a low pH). The volumes given below assume a 1 mg portion. Scale up if more than 1 mg is used.

2. Dissolve the protein sample by adding 100 µL of 4X digestion buffer.

3. Add 12.5 µL of the DTT solution, vortex, spin down to the bottom of the centrifuge tube and incubate at 50˚C in the thermocycler or water bath for 15 min.

4. Remove the sample from the thermocycler, let it cool for several min, add 25 µL of the IAA solution, and incubate in the dark at room temp for 15 min.

5. Add an additional 12.5 µL of DTT solution to the mixture.

6. Add 210 µL of water. Remove 10 µL for analysis by SDS-PAGE to determine the extent of digestion.

7. After 30 min at room temp check pH by spotting 1 µL onto pH paper. If not pH 8.5 adjust by slow addition of 1 N NaOH.

8. Add 40 µL of 1 µg/µL trypsin gold (1:25 ratio of enzyme to substrate), vortex gently to mix, centrifuge to the bottom of the tube and incubate overnight at 37ºC.

9. Remove 10 µL for analysis by SDS-PAGE to determine the extent of digestion.

10. Add 20 µL of neat 88% formic acid to stop the digestion.

11. Sep-Pak clean the sample to remove all salts prior to loading on the polysulfoethyl A column.