

In-solution Digests (revised 8-10-07)

Reagents:

1. 4X digestion buffer: 8 M electrophoresis grade urea, 1.0 M Tris (pH 8.5), 8 mM CaCl₂ and 0.2 M methylamine. (freeze in 50 μ L aliquots)
2. 0.2 M Dithiothreitol (DTT) solution (31 mg into 1.0 mL of water). Make up fresh.
3. 0.5 M Iodoacetamide (IAA) solution (23 mg into 0.25 mL of water). Make up fresh (protect from light).
4. 0.1 μ g/ μ L Promega modified sequencing grade trypsin stock solution. Prepare by dissolving a 20 μ g vial of trypsin (Cat # V5111) in 200 μ L of 1 mM HCl. Store frozen in 20 μ L aliquots for up to 3 months at -20°C, or one year at -70°C. **Do not freeze thaw.**

Protocol:

1. Start with a protein solution of known concentration that is detergent free. Save a portion to run on a gel to assess the extent of digestion.
2. Add the protein solution to a 0.5 mL centrifuge tube. Take the protein sample to dryness in a speed vac. Up to 100 μ g of protein may be digested using the volumes given below, unless high salt concentrations are present that would prevent the sample from being redissolved in 10 μ L of digestion buffer.
3. Thaw the 4X digestion buffer. Add 10 μ L to the dried protein sample and vortex to dissolve.
4. Add 1 μ L 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 1 μ L of the IAA solution, vortex, and incubate in the dark at room temp for 15 min.
5. Add an additional 2 μ L of DTT solution to the mixture, vortex, and incubate for 15 min at room temp.
6. Calculate how much trypsin to add for a final 1:25 enzyme to substrate ratio. For example, if starting with 10 μ g of protein, you would use 4 μ L of 0.1 μ g/ μ L trypsin stock. However, before adding the trypsin, add sufficient water so that the final volume of the digestion mixture is 40 μ L (in this example you would add 22 μ L of water).
7. After addition of water (step 6 above), vortex the sample, and add the trypsin solution.
6. Vortex gently to mix, centrifuge to the bottom of the tube and incubate overnight at 37°C.
7. Add 2 μ L of neat 88% formic acid to stop the digestion.
8. If needed, run equal amounts of undigested and digested samples on a gel to assess the extent of digestion.

9. Freeze at -20°C if LC/MS analysis will not be performed within one week.