SPARC BioCentre
SickKids Proteomics, Analytics, Robotics & Chemical Biology Centre

PROTOCOL

IN-SOLUTION DIGESTION OF PROTEINS

This protocol can be used to digest proteins prior to Mass Spectrometry Analysis.

Procedure:

1. In general, proteins require denaturation and disulfide bond cleavage before enzymatic digestion can go to completion.
2. Dissolve 1–10mg of the target protein in 8M urea, 50mM Tris-HCl (pH 8), 4mM DTT in a reaction volume of up to 1ml (25µl minimum). If your sample does not require denaturation (i.e. cell lysate, immunoprecipitations), then re-suspend your sample in 50-100µL of 50mM NH_4HCO_3 (pH=8.3).
3. Add DTT to final volume of 10 mM.
4. Heat at 60°C for 30 minutes.
5. Allow sample to return to room temperature.
6. Add iodoacetamide (made fresh in ddH_2O) to a final volume of 10mM.
7. Incubate at room temperature in the dark for 15 minutes.
8. Inactivate any remaining iodoacetamide by adding DTT to a final concentration of 40mM.
9. If 8M urea buffer was used, dilute out with 50mM NH_4HCO_3 until concentration is <1M urea.
10. Check the pH of your solution – it should be around 7.5-8.5.
11. Add modified trypsin (MS grade) to a final protease:protein ratio of 1:50-1:100 (w/w).
12. Digest samples O/N @ 37^{o}C.
13. Lyophilize samples, or freeze and drop off at the SPARC facility.