Arizona Proteomics Consortium Protocol
03/07/11

Trypsin digestion of protein solutions

Equipment

heat block or water bath
speed vac
pH paper

Reagents

Sequencing grade modified trypsin (we use Princeton Separations cat # EN-151 or Promega cat # V511)
Nanopure or equivalent H_2O
1 M ammonium bicarbonate pH 7.5-8.0 (Ambic, 79 mg/1 ml ddH_2O)
200 mM dithiothreitol (15.4 mg DTT/500 µl 100 mM Ambic)
200 mM iodoacetamide (18.5 mg IAA/ 500 µl 100 mM Ambic)

Reduction of disulfide bonds and alkylation of cysteine residues

1. Bring sample volume to 80 µl with sufficient 1M Ambic for a final concentration of 100 mM Ambic. Check pH.
2. Add 5 µl 200 mM DTT, incubate at 55ºC for 45 min. Allow tube to cool to room temperature, centrifuge briefly to collect condensate.
3. Add 10 µl freshly prepared 200 mM IAA, incubate at room temperature in the dark for 30 min.

Digestion

1. Add trypsin at a 1:25 to 1:50 trypsin:protein ratio (by weight), mix gently and incubate at manufacturer's suggested temperature for 4 h to overnight.
2. Clean up sample with solid phase extraction (optional).
3. Reduce volume in speed vac to 10 µl, store at -20ºC.