TFE In-solution Digestion Protocol

This procedure is for solution digestion of large amounts of protein (0.5 mg). It can be scaled down for smaller amounts of starting protein.

1. 0.5 mg total protein to 0.5 mL Eppendorf tube. Add 25 μL ammonium bicarbonate stock solution. Add 25 μL TFE denaturation agent. Add 2.5 μL DTT stock solution. Vortex to mix. Heat to at least 60 ºC for 45 minutes-1 hour to denature. (Alternatively use 90 ºC for 20 min)
2. Add 10 μL IAA stock solution. Vortex briefly. Allow to stand at room temperature for 1 hour in the dark (foil covered rack).
3. Add 2.5 μL DTT stock solution to destroy excess IAA. Allow to stand for 1 hour in the dark.
4. Add 300 μL water to dilute denaturant and add 100 μL ammonium bicarbonate stock solution to raise pH. (Note: when handling a large batch of samples, I pre-mix this to reduce pipetting). Trypsin will be denatured or less active if TFE level is greater than 5%. Typical value is 7.5-8.0; add more base if needed.
5. Make fresh stock solution of trypsin in resuspension buffer (see above). Add trypsin stock solution at 1:20 or 1:50 enzyme:substrate. Vortex briefly. Incubate overnight at 37 ºC.
6. Add 2 μL neat formic acid or TFA to lower the pH and stop trypsin activity. Vortex briefly.
7. Digest is ready to dilute and analyze by nanoESI or AP-MALDI. Depending on sample origin, it may be necessary to desalt prior to 2D analysis.

Reagents

Ammonium bicarbonate, reagent grade: e.g., Sigma catalog # A-6141
Dithiothreitol (DTT), >99+%: e.g., Sigma catalog # D-5545
Iodoacetamide (IAA), 97%: e.g., Sigma-Aldrich catalog # I-670-9
Trifluoroethanol (TFE), 99+%: e.g., Sigma-Aldrich catalog # T6,300-2
Sequencing grade trypsin and resuspension buffer: Promega Corporation, catalog # V5111.

Stock solutions

100 mM ammonium bicarbonate: add 100 mL water to 0.7906 g ammonium bicarbonate, store in refrigerator. Will keep (not sure how long), but will slowly degrade as it absorbs carbon dioxide from the air.
200 mM DTT: add 1 mL water to 0.031 g DTT in a 1.5 mL Eppendorf tube. Vortex. Make fresh just prior to use.
200 mM IAA: add 1 mL water to 0.037 g IAM in a 1.5 mL Eppendorf tube. Vortex. Make fresh just prior to use.
Trypsin stock solution: thaw trypsin and resuspension buffer (50 mM acetic acid). Add 20 μL resuspension buffer to trypsin vial (20 μg). Vortex. Make solution just prior to use, allowing 15 minutes for complete resuspension prior to addition to solution containing the substrate.

Reference:

Horth, P.; Miller, C. A.; Preckel, T.; Wenz, C. Efficient fractionation and improved protein identification by peptide offgel electrophoresis. *Mol. Cell. Proteomics* (2006), Vol-5, 1968-1974.