In-Solution Trypsin Digestion

Prepare a precipitated protein sample or an aliquot that has been dried in vacuo. Ensure that chemicals and their final amounts or concentrations are compatible with trypsin activity (see below). For instance, trypsin activity is reduced > 2M urea. For additional information, review the Stability and Reaction Conditions described in the technical documentation for trypsin by Promega. Perform digestions in 1.5 mL Eppendorf protein LoBind microfuge tubes or equivalent.

1. Determine protein concentration and corresponding total protein amount for each sample.
2. Resuspend samples in 25 µl of a solution containing 8 M urea, 0.5 M ammonium biocarbonate, pH 8.0 and 4 mM DTT (or TCEP). Briefly vortex (5 seconds) and centrifuge. Incubate sample at 37°C for 45 min.
3. Let samples cool 5min @ room temperature; add 25 µl of 20mM mM iodoacetamide (or MMTS) in ultrapure water. Briefly vortex and centrifuge. Incubate at room temperature in the dark for 30 minutes. If MMTS is used, it is not necessary to incubate the samples in the dark.
4. Add 50 µl of ultrapure 18 MΩ H_2O to dilute urea below concentrations that would inhibit trypsin (< 2M urea).
5. Reconstitute trypsin in a suitable volume of ultrapure water/20 mM $CaCl_2$. The suitable volume of trypsin is determined by the desired number of micrograms required for the digest; the recommended final trypsin:substrate (wt/wt) ratio is 1:25; the recommended minimum volume of stock trypsin solution (before mixing with protein sample) is 10 µl. Reminder: ensure that the final urea concentration is < 2M. Briefly vortex and centrifuge samples. Incubate at 37°C for 10 – 16 hours (overnight) in a warm air incubator.
6. After overnight digestion, freeze at -80 °C, then speed vacuum to dryness in order to stop trypsin activity. Purify the samples with a suitable solid phase extraction material, for example C18 stage tip, ziptip, etc.

Abbreviations:

TCEP = Tris(2-carboxyethyl)phosphine; DTT = Dithiothreitol; MMTS = Methyl methanethiosulfonate