In-Solution Trypsin Digestion

Reagents: (prepare fresh right before the digestion)

(Use HPLC grade solvents, highest possible grade reagents and MilliQ water for all preparations)

6 M Urea, 50 mM Tris-HCl, pH 8.0

200 mM DTT, 50 mM Tris-HCl, pH 8.0

200 mM Iodoacetamide, 50 mM Tris-HCl, pH 8.0

50 mM Tris-HCl, 1 mM $CaCl_2$, pH 7.6

Trypsin solution (0.2 μg/μl) : Reconstitute or dilute trypsin stock in resuspension buffer (50 mM acetic acid), keep on ice before use. (Sequencing Grade Modified Trypsin, Promega Cat# V5111)

Procedure:

1. Reconstitute the target protein (0.1-1 mg) in 100 μl of 6 M Urea, 50 mM Tris-HCl, pH 8.0. (The amount of protein depends on how complex your protein mixture is, you need to obtain a final concentration of >10 fmol/μl of each protein in the protein mixture after you finish the final step of the digestion)
2. Add 5 μl of 200 mM DTT/ 50 mM Tris-HCl, pH 8.0, and incubate the mixture for 1 h at room temp.
3. Add 20 μl of 200 mM Iodoacetamide/ 50 mM Tris-HCl, pH 8.0, gentle vortex, and incubate the mixture for 1 h at room temp in dark.
4. Add 20 μl of 200 mM DTT/ 50 mM Tris-HCl, pH 8.0 to consume any unreacted iodoacetamide. Incubate the mixture for 1 h at room temp in dark.
5. Add 775 μl of 50 mM Tris-HCl, 1 mM $CaCl_2$ (pH 7.6) to reduce the urea concentration to ~0.6 M.
6. Add Trypsin solution to a final ratio of 1:50 (w/w, trypsin : protein). Gentle vortex and incubate at 37^{o}C for 16-20 h.
7. Add formic acid to adjust pH to 3-4. Test pH by placing 1-μl aliquots onto a pH paper. Store at -20^{o}C.

Reference:

1. Promega Sequencing Grade Modified Trypsin Product Information Sheet.
2. Kinter, M., and Sherman, N. E. 2000, Protein sequencing and identification using tandem mass spectrometry. John Wiley & Sons, Inc. pp.161-163