IN-SOLUTION TRYPSIN DIGESTION

Reagents:

NOTE: To be freshly prepared before the digestion procedure

- 7-8M Urea, 50 mM – 100 mM NH_4HCO_3, pH 8.0
- 200 mM DTT, 50 mM NH_4HCO_3, pH 8.0
- 200 mM Iodoacetamide (IAA), 50 mM NH_4HCO_3, pH 8.0
- 50 mM NH_4HCO_3, 1 mM $CaCl_2$, pH 7.6
- Trypsin solution: Reconstitute or dilute Trypsin stock in resuspension buffer (50 mM acetic acid), keep on ice before use. (MS Grade- Modified Trypsin)

Procedure:

1. Reconstitute the target protein (0.1-1 mg) in 100 ul of 7-8 M Urea, 50 mM NH_4HCO_3, pH 8.0.
2. Add 5 ul of 200 mM DTT/ 50 mM NH_4HCO_3, pH 8.0, and incubate the mixture for 1 hour at RT. **[10mM DTT, final concentration]**
3. Add 20 ul of 200 mM Iodoacetamide/ 50 mM NH_4HCO_3, pH 8.0, gently vortex, and incubate the mixture for 1 hour at room temp in dark. **[50 mM IAA, final concentration]**
4. Add 20 ul of 200 mM DTT/ 50 mM NH_4HCO_3, pH 8.0 to consume any unreacted iodoacetamide. Incubate the mixture for 1 hour at room temp in dark.
5. Add 50 mM NH_4HCO_3, 1 mM $CaCl_2$ (pH 7.6) to reduce the urea concentration to ~ 0.5 M.
6. Add Trypsin solution to a final ratio of 1:50 (w/w, trypsin: protein). Gently vortex and incubate at 37ºC for 16-20 hours.
7. Add formic acid to adjust pH to 3-4. Test pH by placing 1ul aliquot onto a pH paper. Store at -20ºC (optional).
8. Desalt the tryptic peptides using C18 spin columns.