In solution Trypsin Digestion Protocols

Trypsin Gold, Mass Spectrometry Grade (Promega, 100µg, cat.# V5280)

1. Reconstitute or dilute the target protein in 8M urea, 50mM Tris-HCl (pH 8), 10mM DTT (digestion buffer). The recommended protein starting amount is 50 µg.
   Final urea concentration after sample dilution should be about 6M.

   (For example 100µL final reconstituted sample volume:
   Mix 25µL protein stock (1-2mg/mL) with 75µL of digestion buffer)

2. Incubate at 37°C for 60 minutes.
3. Add Chloroacetamide (CAA) from the 500 mM stock solution to a final concentration of 50 mM. Mix and leave in the dark at RT for 30 min. CAA stock solution must be prepared fresh and used immediately. Prepare a 500 mM CAA stock solution by dissolving 0.023 g of CAA in 0.5 mL of 50mM Tris-HCl (pH 8).
4. Add 50mM Tris-HCl (pH 8), 1mM CaCl2, until the urea concentration is 1M. !!!!
5. Add Trypsin Gold to a final protease: protein ratio of 1:20 (w/w).
6. Incubate at 37°C overnight.
7. After overnight digestion, acidify the sample by adding 100% formic acid to sample from the step 6. to a final concentration of 2% (vol/vol) to a pH of 2–3 to stop the enzymatic activity.
8. Desalt peptides using **desalting spin columns** (https://www.thermofisher.cn/order/catalog/product/89851#/89851).
9. Lyophilize eluted peptides (from the spin column protocol) using SpeedVac Vacuum Concentrator.
10. Store dry peptides at -20C
11. Resuspend peptides in 0.1% Formic acid just before LC-MS