In-Solution Tryptic Digestion Protocol

Note:

It is particularly important during this procedure to avoid contaminating your samples with protein. The followings are some of very important practices to avoid contaminations.

1. *All sample preparation steps prior to trypsin digestion should be done in a BSC or laminar flow hood.*
2. *To avoid Keratin, a human skin protein, contamination you should wear nitrile (not latex) gloves when handling any instruments or containers that will come into contact with your sample or solutions.*

Materials:

- 50 mM Ammonium bicarbonate
- Rapigest solution (Make up Rapigest with 50 mM NH_4HCO_3)
- Dithiothreitol (DTT)
- 200 mM Iodoacetamide (IAM) in 50 mM NH_4HCO_3
- Trypsin

Procedure:

Denaturation/ Reduction/Alkylation of Proteins

1. Make sure proteins are in an appropriate buffer (20mM Tris-HCl, or 20mM Ammonium Bicarbonate, pH 7.5-8). Protein concentration should be around 1 μg/μl. (Bradford assay should be performed to determine protein concentration of each solution)
2. Any commonly used denaturants can be used to increase the solubility of proteins. However, we suggest Waters Rapigest solution for this purpose. Add Rapigest solution to have 0.1% final concentration.

3. Incubate at 37°C for 10 min while shaking. Spin down.

4. Prepare 100 mM DTT using 50mM NH_4HCO_3 and add DTT to each sample (Final DTT concentration should be around 10 mM)

5. Heat solution at 75 °C for 15 minutes, let cool and spin down.

6. Add iodoacetamide (freshly prepared) to each solution, make up the final iodoacetamide concentration to 20mM, and incubate at room temperature for 30 min in the dark

Digestion of Proteins

1. Add trypsin solution to each sample at 1:50 trypsin: protein concentration. Incubate at 37°C for 3 hrs or overnight while shaking.

2. Quick spin to collect condensate to bottom of vials. Add TFA and CH_3CN to give 0.5% and 2% by volume respectively and incubate at room temperature for 5 min.

3. Spin down for 10 minutes. keep the supernatant.

4. Perform Sep-Pak desalting or equivalent for sample cleanup.

5. Place sample vials in the SpeedVac. With the cold trap on, spin them until the liquid has evaporated. Re-suspend in LC buffer (0.2% formic acid, 2% acetonitrile in water)

This Protocol is adapted from "Large-scale quantitative assessment of different in-solution protein digestion protocols reveals superior cleavage efficiency of tandem Lys-C/trypsin proteolysis over trypsin digestion" by Glatter, T., Ludwig, C., Ahrné, E., Aebersold, R., Heck, A. J., & Schmidt, A. (2012), *Journal of proteome research*, *11*(11), 5145-5156.