In-solution Digestion of Proteins

Boisvert Lab, December 2020

1. Solubilize proteins directly in a solution of 8 M urea, 1 M NH_4HCO_3, 20 mM HEPES pH 8.0 (lysis buffer)(in MS-grade water). How much sample to use depends on the amount of proteins, and the purpose of your experiment. For a total cell extract, resuspend directly the cell pellet coming from a 100mm petri dish of confluent cells (~2 mg of total protein) in 500 µl of lysis buffer. Sonicate on ice (12 cycles of 5 sec PULSE /5 sec OFF, intensity of 20-25%), centrifuge 16,000 x g for 10 min at 4ºC and transfer the supernatant to a new lobind tube. Quantify your cell extract using BCA kit (compatible with 3M urea, so do not forget to dilute your sample). Generaly speaking, transfer 50 µl of cell extract containing up to 50 µg of total proteins to a new lobind tube and proceed to the next step.

2. Add DTT (5 mM final concentration). Boil at 95ºC for 2 minutes, then incubate at RT for 30 min.

3. Add chloroacetamide (7.5 mM final concentration). Incubate at RT for 20 min in the dark.

4. Add 150 µl of 1 M NH_4HCO_3 (in MS-grade water) to the mixture so that the final concentration of urea is reduced to 2 M.

5. Add 1µg trypsin and incubate at 30ºC overnight.

6. Acidify the sample to a final concentration of 0.2 % TFA.

7. Clean up using a ZipTip.

Notes

1. Remember to wear gloves at all times during the procedure. Don't scratch your head, armpits, genital, or any part of your body during the procedure. Keratin contamination is less an issue after peptide digestion, as whole keratin proteins will not interfere with MS analysis.

2. When you make any stock solutions, avoid using any spatula that have not been cleaned thoroughly by water and ethanol. In fact, we routinely forgot using spatula at all, and tip the solid chemical into clean eppendorf tubes.

3. Peptides tend to stick to plastic surfaces on storage. We use 1.5 ml lobind tubes that are designed for storing peptides at low concentration.

Adapted from Dr Angus Lamond lab.