

Sample Preparation Protocol for Global Proteomics

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1. Resuspend the protein pellet in 10uL of the denaturation solution (8M Urea + 10mM DTT in 10mL water) up to 100ug of protein. Best to freeze the pellet until ready to trypsin digest.
2. Incubate at 37°C for 1.5 hours.
3. Add 2uL of a 100mM Ammonium bicarbonate solution.
4. Add 10uL of the freshly prepared cocktail (shown below) to each sample and incubated at 37°C for 1.5 hours.
 - a. 195uL Acetonitrile (97.5% of solution)
 - b. 1uL TEP (0.5% of solution)
 - c. 4uL 2-Iodoethanol (2% of solution)
5. After incubation in cocktail, put sample into the speed vac overnight or until completely dry.
6. Resuspend the dry sample (you will now see dry white Urea in the tube) in 80uL of a 100mM Ammonium carbonate solution.
7. Add appropriate gram amount of enzyme (Trypsin solution digest ratio: 1ug enzyme to 50ug protein). Digestion will take place at 37°C for at least 12 hours.
8. Post-digestion, stop the enzymatic reactions (and ionize the resulting tryptic peptides) by adding 1uL of trifluoroacetic acid to the sample.
9. Run sample over C18 column for buffer exchange.
10. Equilibrate column by adding 500ul 100% ACN to column, then spin at approx. 100Xg for 2 minutes.
Then add 500ul H₂O, and spin at approx. 100Xg for 2 minutes.
11. Add Sample, spin at approx. 100Xg for 2 minutes.
12. Rinse column by adding 200ul 0.1% TFA in H₂O, and then spin at approx. 100Xg for 2 minutes. Repeat.
13. Elute sample by adding 50ul of 80% ACN with 0.1% TFA, then spin at approx. 100Xg for 2min. Repeat.
14. Lyophilize sample and resuspend in 0.01% TFA in H₂O or buffer of choice.
15. Run LCMS.

