**Sonication**

1. Add 100 µl 6M urea in 50 mM NH4HCO3 (ammonium bicarbonate)
2. Sonicate each sample for 10s, chilling in ethanol + dry ice for 5s and storing on wet ice between sonications. Repeat twice for each sample (total of 3 times).
3. Between samples, rinse the sonicating probe with ethanol and nanopure water.
4. Store at -80°C if not moving directly to sample digestion.

**Protein quantification – BCA Assay (Pierce)**

*This can be done prior to trypsin digestion or in parallel.*

1. Aliquot 11 µl of sonicated sample to a clean tube and add 22 µl NH4HCO3 to dilute urea. Follow the microplate protocol for BCA using 10 µl of sample and Genn’s dilution curve (below). Lysis buffer = 50 mM NH4HCO3 with 6M urea diluted 1:2.

|  |  |  |  |
| --- | --- | --- | --- |
| Vial | BSA conc. (µg/µl) | Vol. lysis buffer (µl) | Vol. BSA & dilution |
| B | 1.5 | 125 | 375, stock |
| C | 1.0 | 325 | 325, stock |
| D | 0.75 | 175 | 175, B |
| E | 0.5 | 325 | 325, C |
| F | 0.25 | 325 | 325, E |
| G | 0.125 | 325 | 325, F |
| H | 0.025 | 400 | 100, G |

1. Use BCA protocol equations to calculate the sample volume containing 100 µg of protein. Make sure to correct for any dilutions.

**Mini-Trypsin digestion**

*This digestion is for total starting volume of 100 µl. You can either aliquot the volume to equal 100 µg of protein to a new tube and add additional 6M urea in 50 mM NH4HCO3 (Step 9 - save remaining lysed cells/proteins in -80°C freezer), or digest your entire sample if it is <100 µg protein dissolved in 100 µl total volume.*

1. Add 6.6 µl of 1.5 M Tris pH 8.8
2. Add 2.5 µl 200 mM TCEP and vortex
3. Test pH of samples to make sure they are still basic.
4. Incubate samples 1 hour at 37°C
5. Add 20 µl of 200 mM iodoacetamide (IAA)
6. Incubate 1 hour, room temperature, in dark
7. Add 20 µl 200 mM diothiothreitol (DTT, freshly made or frozen), vortex
8. Incubate 1 hour, room temperature
9. Aliquot the volume equal to 100 µg of protein to a new tube (save remainder at -80°C)
10. OPTIONAL: Add ApoA1 to each sample (dilute 1.1 µl 2200 ng/µl stock in 48.9 µl of 50mM NH4HCO3 to make 50 ng/µl) to make 1:300 ratio of ApoA1:total protein.
    1. For 100 µg total protein in a sample, add 333 ng of ApoA1 by adding 6.7 µl of 60 ng/µl ApoA1 solution.
11. Add LysC at a 1:30 enzyme:protein ratio to each sample. (LysC cleaves at C-terminus of R and K, like trypsin, but works well in 6M urea.)
12. Incubate 1 hour, room temperature
13. Add 800 µl 25 mM NH4HCO3 and 200 µl HPLC grade methanol to each tube.
14. Prepare the number of trypsin bottles needed (you will want 5 µg of trypsin for each sample, or 1 µg trypsin: 20 µg protein). Add 20 µl water or trypsin buffer to each bottle of trypsin and vortex lightly. Aliquot 5 µl of trypsin to each sample for 100 µg of sample (1:30 enzyme:protein).
15. Incubate overnight at room temperature or for 4 hours at 37°C.
16. Evaporate samples at 4°C to near dryness on speed vacuum (<20 µl). Store at -80°C.

**Desalting**

Solvent A = 60% acetonitrile + 0.1% trifluoroacetic acid

Solvent B = 5% acetonitrile + 0.1% trifluoroacetic acid

1. Reconstitute samples in 100 µl solvent B. Ensure pH<2, if it isn’t, add 10 µl increments of 10% formic acid until pH<2.
2. Prepare spin columns – see table below for column choice.

|  |  |  |  |
| --- | --- | --- | --- |
| Column type | Sample capacity(µg) | Elution volume (µl) | Bed volume (µl) |
| UltraMicro Spin | 0.03-30 | 5-25 | 50 |
| MicroSpin | 0.05-60 | 10-50 | 100 |
| MacroSpin | 0.03-300 | 50-150 | 300 |
| 96-well MiniSpin | 0.03-100 | 30-50 | 100 |
| 96-well MACROspin | 0.03-300 | 60-150 | 300 |

1. Wash column: Add 200 µl solvent A to columns, spin for 2000 rpm 3 minutes (repeat 3 times)
2. Equilibrate column: Add 200 µl solvent B to columns, spin for 2000 rpm for 3 minutes (repeat 2 times)
3. Load protein on column: Add 100 µg of protein digest (1 sample per column). Spin at 3000 rpm for 3 minutes. Collect flow-through, put back on column and spin again.
4. Wash salts through column: Wash columns with 200 µl solvent B, spinning at 3000 rpm for 3 minutes (repeat twice).
5. Elute peptides: Transfer columns to clean collection tubes. Add 100 µl solvent A, spin 3000 rpm for 3 minutes (repeat once).
6. Evaporate samples to near dryness (can be at approximately room temperature).
7. Reconstitute peptides in 100 µl 2-5% ACN + 1% formic acid. Store at -80°C.