Required material/equipment/chemicals/reagents

- Urea (U0631-500G, Sigma)
- Ammoniumbicarbonate (Sigma, A6141- 500g)

Required working reagents/buffers for protein extraction:

- Lyses buffer: 8 M Urea, 0.1M Ammoniumbicarbonate
- Vial Tweeter (Ultrasonicator, Hielscher)
- BCA assay (e.g. Pierce, 23225) or similar reagents for protein measurement

Experimental - Protein extraction from whole cells

1. Dissolve cell pellet in a lysis buffer volume two times bigger than pellet
2. Vortex for 10 sec
3. Ultra sonicate samples for 2 x 10 sec with vial Tweeter (put on ice between steps)
4.Rotate 5 minutes at 25°C at 1,400 rpm
5. Spin down at 10,000 rpm for 10 sec
6. Use a BCA/Bradford assay (Pierce) to measure protein concentration from the supernatant. Use BSA dissolved in lysis buffer to generate calibration curve.
7. For standard protein digests use 100ug protein, for subsequent phosphopeptide enrichment or peptide fractionation use at least 1mg – better more - of total protein.
.