

Methanol-Chloroform Precipitation

- For standard samples you want to add 4 parts methanol, 3 parts protein sample resuspended in water and 1 part chloroform.
- If sample has a high concentration of phospholipids, you want to add 2 parts chloroform
- To 300 μ l protein sample, add 400 μ l methanol and vortex.
- Then add 100 μ l (or 200 μ l if high phospholipid content) chloroform in the hood to your sample and vortex vigorously.
- Centrifuge sample at 14K, 4°C, 5 minutes.
- You should see three phases: the clear upper aqueous phase, the cloudy precipitated protein interphase and the clear lower chloroform phase.
- Carefully remove and discard the top aqueous phase without disrupting the protein interphase.
- Add 300 μ l methanol to the remaining phases and vortex.
- Centrifuge sample at 14K, 4°C, 5 minutes to pellet protein.
- Remove supernatant from pellet and speed vacuum pellet for 10 minutes with no heat.
- Pellet is now ready for digestion or protein quantitation.