

Methanol/Chloroform Protein Precipitation

- Method to process 100uL of protein sample; it can be scaled up or down.
 1. Add 400uL of Methanol to a sample of 100uL volume.
 2. Vortex well.
 3. Add 100uL of Chloroform.
 4. Vortex well.
 5. Add 300uL of ddH₂O. Sample should look cloudy.
 6. Vortex well.
 7. Spin 2 min. @ 14,000 g
 8. Pipette off the top aqueous layer. DO NOT lose protein - protein exists between layers and may be visible as a thin wafer.
 9. Add 400uL of Methanol.
 10. Vortex well.
 11. Spin 3min. @14,000 g
 12. Pipette as much Methanol as possible from the tube without disturbing the pellet.
 13. Speed-Vac to dryness but avoid drying too long as this makes the pellet harder to re-solubilize.
 14. Solubilize the pellet in buffer appropriate for downstream process.