

Methanol Chloroform Precipitation of Proteins

This is a method to precipitate proteins that are not adequately precipitated by TCA/NaDOC.

1. To 200 μ l protein sample, add 480 μ l MeOH (filtered), 160 μ l CHCl_3 (filtered, add under the hood). Mix sample by vortexing. Add 640 μ l ddH₂O, vortex and spin 5 min at 14,000rpm.
2. Suck off the upper layer (the proteins are between the two liquid phases: top-MeOH/H₂O, bottom- CHCl_3) leaving a small amount of upper phase behind (use a thin gel-loading tip).
3. Add 300 μ l MeOH, vortex and spin 30 min, 14,000 rpm, at 4°C.
4. Suck off supernatant completely (carefully) and air dry protein pellet.
5. Resuspend protein pellet in SDS sample buffer (solution A and solution B).