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METHANOL/CHLOROFORM/WATER PROTEIN PRECIPITATION

(Wessel, D., and Fugge, U.I. A method for the quantitative recovery of protein in dilute solution in the presence of detergents and lipids. Analytical Biochemistry (1984), 138; 141-143)

1. Pipet 100 ul of protein sample into a 1.5 ml eppendorf tube
2. Add 400 ul of 100% MeOH; vortex well
3. Spin briefly to collect the sample
4. Add 100 ul of Chloroform; vortex well (use 200 ul of chloroform for high phospholipid conc)
5. Spin briefly to collect the sample
6. Add 300 ul of mQwater (for phase separation); vortex well
7. Spin at 13k x rpm, 1 min, RT; (protein should appear at interface)
8. Remove and discard the upper layer (CAREFUL , DON'T REMOVE PROTEIN!)
9. Add 300 ul of MeOH to the tube
10. Vortex well and spin at 13k x rpm for 2 minutes, RT (this causes the protein to form a pellet)
11. Remove the supernatant
12. Air dry the pellet (or under a stream of nitrogen or argon)
13. Resuspend in buffer of choice

This procedure can be adapted to larger volumes. For example, 1 ml sample is precipitated by adding 4 ml MeOH, 1 ml chloroform and 3 ml water. After centrifugation and removing the upper layer, the lower layer and interface proteins are transferred to 1.5 ml eppendorf tubes and the procedure is applied as described above. (The ratio of protein:MeOH: chloroform: water is 1:4:1:3).

Removes SDS, TX-100, salts, beta-mercaptoethanol.