

Precipitation techniques

Purpose: to concentrate the sample and remove small interfering species, such as salts & detergents, for downstream applications e.g. 2D gels.

For small amounts of protein (< low microgram) it may be necessary to add a carrier protein such as insulin.

Acetone Precipitation Protocol

1. Cool the required volume of acetone to -20°C.
2. Place protein sample in acetone-compatible tube.
3. Add four times the sample volume of cold (-20°C) acetone to the tube.
4. Vortex tube and incubate for 60 minutes at -20°C.
5. Centrifuge 10 minutes at 13,000-15,000 x g.
6. Decant and properly dispose of the supernatant, being careful to not dislodge the protein pellet.
Optional: If additional cycles of precipitation are necessary to completely remove the interfering substance, then repeat steps 2-5 before proceeding to step 7.
7. Allow the acetone to evaporate from the uncapped tube at room temperature for 30 minutes. Do not over-dry pellet, or it may not dissolve properly.
8. Resuspend in appropriate buffer.

TCA Precipitation Protocol

1. Add an equal volume of 20% TCA (trichloroacetic acid) to protein sample.
2. Incubate 30 min on ice.
3. Spin in microfuge at 4 deg. For 15 min.
4. Carefully remove all supernatant.
5. Add ~300 ul cold acetone and spin 5 min at 4 degrees.
6. Remove supernatant and dry pellet.
7. Resuspend samples in desired buffer.

Warning: TCA is a strong acid and should be handled with care

Chloroform/Methanol Precipitation

1. To sample of starting volume 100 ul
2. Add 400 ul methanol
3. Vortex well
4. Add 100 ul chloroform
5. Vortex
6. Add 300 ul H₂O
7. Vortex
8. Spin 1 minute @ 14,000 g
9. Remove top aqueous layer (protein is between layers)
10. Add 400 ul methanol
11. Vortex
12. Spin 2 minutes @ 14,000 g
13. Remove as much MeOH as possible without disturbing pellet
14. Speed-Vac to dryness
15. Bring up in 2X sample buffer for PAGE

Reference: Wessel, D. and Flugge, U. I. Anal. Biochem. (1984) 138, 141-143

