Protein precipitation

Acetone Precipitation
Most universal

Materials:
• Sample of interest
• Cold (-20°C) acetone, a volume four times that of the protein samples to be precipitated
• Centrifuge tube
• Centrifuge

Method:
1. Cool the required volume of acetone to -20°C.
2. Add four times the sample volume of cold (-20°C) acetone to the tube.
3. Vortex tube and incubate for 60 minutes at -20°C.
4. Centrifuge 10 minutes at 13,000-15,000 x g.
5. Decant and properly dispose of the supernatant, being careful to not dislodge the protein pellet.
6. Add 0.5 ml cold acetone, vortex briefly.
7. Centrifuge 10 minutes at 13,000-15,000 x g.
8. Aspirate acetone, avoiding pellet with care.
9. 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.

Chloroform/Methanol Precipitation
Useful method for removal of salt and detergents

Materials:
• Samples of interest
• Methanol
• Chloroform
• Centrifuge tube
• Centrifuge and rotor for the tubes used, minimum 13,000 x g required

Method:
1. To 100uL protein sample in a 1.5mL eppendorf tube:
2. Add 400 uL methanol and vortex thoroughly.
3. Add 100 uL chloroform and vortex.
4. Add 300 uL H2O—mixture will become cloudy with precipitate—and vortex.
5. Centrifuge 5 minute at 14,000 x g. Result is three layers: a large aqueous layer on top, a circular flake of protein in the interphase, and a smaller chloroform layer at the bottom.
6. Remove top aqueous layer carefully, trying not to disturb the protein flake.
7. Add 400 uL methanol and vortex.
8. Centrifuge 5 minutes at 14,000 x g. which will slam dandruffy precipitate against the tube wall.
9. Remove as much methanol as possible. Be careful, because the pellet is delicate. You should be able to remove all but a few uL of methanol with care, which will speed drying.
10. Briefly dry the pellets in vacuum centrifuge.
Ethanol Precipitation
Useful method to concentrate proteins and removal of Guanidine Hydrochloride before SDS-PAGE

Materials:
- Samples of interest
- Ethanol (cold)
- Centrifuge tube
- Centrifuge

Method:
1. Add to 1 volume of protein solution 9 volumes of cold ethanol 100%.
2. Mix and keep at least 60 min. at -20°C. (Suggestion: leave ON).
3. Centrifuge 10 minutes at 15,000 x g.
4. Carefully discharge supernatant and retain the pellet; dry tube by inversion on tissue paper (pellet may be difficult to see).
5. Wash pellet with cold ethanol (keep at -20°C).
6. Vortex and repellet samples for 10 min at 15,000 x g.
7. Aspirate ethanol, avoiding pellet with care
8. Dry samples under vacuum (speed vac) or dry air to eliminate any ethanol residue (smell tubes).

TCA Precipitation

Materials:
- Samples of interest
- TCA = trichloroacetic acid, 100% solution stored at 4°C
- Acetone (cold)
- Centrifuge tube
- Centrifuge

Method:
1. Add 100% TCA from cold stock solution to the sample(s) of interest such that the final mixture is 20% TCA
2. Vortex and incubate for 30 minutes on ice.
3. Centrifuge 10 minutes at 13,000-15,000 x g. at 4 °C.
4. Aspirate the supernatant carefully, to not disturb the pellet (may be seen on the side or bottom of the tube).
5. Add 0.5 ml cold acetone, vortex briefly.
6. Centrifuge 10 minutes at 13,000-15,000 x g. at 4 °C.
7. Aspirate acetone, avoiding pellet with care.
8. 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.