PROTOCOL

ACETONE PRECIPITATION OF PROTEINS

This protocol can be used to precipitate proteins from solutions where heavy detergents were used to prepare protein extracts.

Required materials:

- Cold (-20°C) acetone (volume = 4X that of the protein samples to be precipitated)
- Centrifuge tubes made of acetone-compatible polypropylene and able to hold 5X sample volume
- Centrifuge and rotor for the tubes used (min speed 18000 x *g*)
- 1M Urea/50 mM NH_4HCO_3

Procedure:

1. Cool required volume of acetone to -20°C.
2. Place protein sample into acetone-compatible tube.
3. Add 4 fold volume of cold acetone to protein mixture.
4. Vortex and incubate at -80°C for 1 hour (or overnight)
5. Centrifuge @ 18,000*g* for 10 minutes
6. Decant and properly dispose of supernatant, keeping in mind to not disturb the protein pellet.
7. Allow pellet to air dry for 10-30 minutes in uncapped tube. Do not over-dry the pellet as it may not re-dissolve properly.
8. Re-suspend the pellet in 50-100µL 1M Urea/50mM NH_4HCO_3.
9. Bring sample to SPARC facility OR, proceed with digestion.