Acetone precipitation

Adapted from Thermo Scientific Tech Tip #49.

Introduction

Protein samples commonly contain substances that interfere with downstream applications. Several strategies exist for eliminating these substances from samples. Small soluble substances may be removed and the samples exchanged into appropriate buffers by dialysis or gel filtration (desalting columns). A variety of Thermo Scientific dialysis and desalting products are available for performing such buffer exchanges with small or large sample volumes (see Related Products).

Another strategy for removing undesirable substances is to add a compound that causes protein to precipitate. After centrifugation to pellet the precipitated protein, the supernatant containing the interfering substance is removed and the protein pellet is re-dissolved in a buffer that is compatible with the downstream application. Several methods for protein precipitation are described in the literature. A popular method using acetone is presented here.

Important notes

Precipitation has an advantage over dialysis or desalting methods in that it enables concentration of the protein sample as well as purification from undesirable substances.

One disadvantage of protein precipitation is that proteins might denature, making the pellet difficult to re-solubilize. Therefore, use precipitation only for downstream applications in which solvents that aid in re-solubilizing the sample will be used (e.g., 2-D electrophoresis sample buffer, SDS-PAGE sample buffer, Pierce® BCA Protein Assay Reagent). For precipitation before performing a BCA Protein Assay, see Thermo Scientific Tech Tip #8: Eliminate interfering substances from samples for BCA Protein Assay.

A single precipitation may not be sufficient to remove all types and concentrations of interfering contaminants. In such cases, repeated precipitation may be performed. However, because some sample loss will accompany each cycle of precipitation, use only the number of cycles necessary for the application.

Reagents

Cold (-20°C) acetone, a volume four times that of the protein samples to be precipitated

Centrifuge tube, made of acetone-compatible polypropylene and able to hold five times the sample volume

Centrifuge and rotor for the tubes used, minimum 13,000 × g required

Procedure

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 × g.
6. Decant and properly dispose of the supernatant, being careful to not dislodge the protein pellet.
7. Optional: If additional cycles of precipitation are necessary to completely remove the interfering substance, then repeat steps 2-5 before proceeding to step 7.
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.
9. Add buffer appropriate for the downstream process and vortex thoroughly to dissolve protein pellet.