mixture was incubated at 25 °C for 25 minutes, and then 100 µl of the mixture was injected into the column and the time of its elution noted by following the A_{280} of the eluant as it eluted from the column. The molecular mass of the complex was determined by fitting its elution time to the calibration curve constructed for the molecular mass standards. The protein in the peak fractions was analysed by Tris-glycine SDS PAGE (Section 2.7.3).

2.23 Glutaraldehyde cross-linking of the Rsd protein

A sample of the purified Rsd protein (Section 2.14) was dialysed against 1000 volumes of 1 × phosphate buffered saline (PBS) overnight. Different concentrations of glutaraldehyde: 0.025 % (v / v), 0.05 % (v / v), and 0.1 % (v / v) were prepared from a 5 % (v / v) stock solution. 10 µg Rsd was incubated with 1 µl of each of the different glutaraldehyde concentrations in a final volume of 50 µl to give final glutaraldehyde concentrations of 0.0005 % (v / v), 0.001 % (v / v), and 0.002 % (v / v). The volume of the mixture was made up to 50 µl with bicine buffer (50 mM bicine (pH 8.5), 0.4 M NaCl, 0.1 mM DTT). The mixtures were left for 20 minutes at 25 °C, after which the reactions were stopped by the addition of 2 µl of ethanolamine. The proteins in the mixtures were precipitated by the addition of 4 volumes of acetone to the mixture, after which the mixture was incubated at –20 °C for 90 minutes. The samples were centrifuged at 10 000 × g for 20 minutes. The protein pellet was resuspended in 10 µl of 1 × sample buffer, and the mixture loaded onto a denaturing Tris-tricine polyacrylamide gel, with a 4 % (w / v) polyacrylamide stacking gel and a 8 % (w / v) polyacrylamide resolving gel (Section 2.7.4). The gel was run until the tracking dye reached the bottom of the gel, and the bands due to the proteins were visualised by staining with Coomassie brilliant blue stain. The gel was destained and scanned using a densitomter (Bio-Rad). The relative mobility values of bands due to proteins were determined using the Quantity One software (Bio-Rad).

To determine the molecular mass values of the cross-linked species, the relative mobility values of the protein standards were measured (Table 2.9). A calibration curve was constructed by plotting the relative mobility values of the bands

due to the molecular mass standards as a function of the logarithm$_{10}$ of their molecular mass. Thus, the molecular mass of protein bands due to cross-linked Rsd could be determined by fitting their relative mobility values to the (R_f : $\log_{10} M_r$) calibration curve constructed using the standards.

TECHNICAL RESOURCE

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Acetone precipitation of proteins

TR0049.0

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). Pierce offers a variety of dialysis and desalting products for performing such buffer exchanges with small or large sample volumes (see Related Pierce 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 buffer compatible with the downstream application.

A variety of 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 may be denatured, 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, BCA™ Reagent). For precipitation before performing a BCA™ Protein Assay, see the Tech Tip "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.

Materials Required

- Cold (-20°C) acetone, a volume four times that of the protein samples to be precipitated
- Centrifuge tube, made of acetone-compatible material such as polypropylene and able to hold five times the sample volume
- Centrifuge and rotor for the tubes used, minimum 13,000 x *g* required

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. Add buffer appropriate for the downstream process and vortex thoroughly to dissolve protein pellet.

Related Pierce Products

89849	**Protein Desalting Spin Columns,** 25 columns
20439	**D-Salt™ Excellulose™ Desalting Columns,** 5 x 2 ml columns
66380	**Slide-A-Lyzer® Dialysis Cassette, 10 MWCO, 0.5-3ml,** 10 units
89865	**2D Sample Prep for Soluble Protein,** Sufficient reagents for 25 applications
23215	**Compat-Able™ Protein Assay Preparation Reagent Set,** sufficient reagents to pre-treat 500 samples to remove interfering substances before total protein quantitation