

## Protocol for analytical ultracentrifugation sample preparation:

1. Provide partial specific volume ( $v$ -bar) of the proteins. If  $v$ -bar is not available, send complete amino acid sequence of your protein via e-mail. Please type the sequence into the body of the e-mail rather than sending it as an attached file.
2. Provide minimum 250  $\mu$ L of each sample to be analyzed for equilibrium runs and 1 mL for velocity runs and at least 5 mL of buffer.
3. For accurate determination of molecular weight or oligomeric state, provide three different concentrations for each sample. An example of an ideal concentration series is 0.25 mg/mL, 0.50 mg/mL, and 1.00 mg/mL. Ensure that the absorbance at either 230 nm or 280 nm (or, whatever other wavelength) is not less than 0.25 at the lowest concentration, and does not exceed 1.00 at the highest concentration. Concentrations as low as 1  $\mu$ g/mL are acceptable only if the absorbance at 230 nm is at least 0.30. Please make your own dilutions.
4. Try to avoid using DTT, 2-mercaptoethanol, and protease inhibitors. If DTT is absolutely required to maintain sample stability, do not exceed 5 mM concentration. For ATP or ADP try to use less than 0.3 mM.
5. The reference buffer should contain all components that are in the sample minus the protein.
6. Provide us with the samples, buffers, and filled form.
7. Samples will be stored at 4°C and run at 4°C, unless otherwise specified.

### Acceptable buffer components:

1-Propanol	2-Propanol	Acetic Acid	Acetone
Ammonium chloride	Ammonium hydroxide	Ammonium sulfate	Barium chloride
Cadmium chloride	Cadmium sulfate	Calcium chloride	Cesium chloride
Citric acid	Cobaltous chloride	Creatinine	Cupric sulfate
EDTA disodium	Ethanol	Ethylene glycol	Ferric chloride
Formic acid	Fructose	Glucose	Glycerol
Guanidine hydrochloride	Hydrochloric acid	Inulin	Lactic acid
Lactose	Lanthanum nitrate	Lead nitrate	Lithium chloride
Magnesium chloride	Magnesium sulfate	Maltose	Manganous sulfate
Mannitol	Methanol	Nickel sulfate	Nitric acid
Oxalic acid	Phosphoric acid	Potassium bicarbonate	Potassium biphthalate
Potassium bromide	Potassium carbonate	Potassium chloride	Potassium chromate
Potassium dichromate	Potassium ferricyanide	Potassium ferrocyanide	Potassium hydroxide
Potassium iodide	Potassium nitrate	Potassium oxalate	Potassium permanganate
Potassium PO <sub>4</sub> di-basic	Potassium PO <sub>4</sub> mono-basic	Potassium sulfate	Potassium thiocyanate
Procaine hydrochloride	Propylene glycol	Silver nitrate	Sodium acetate
Sodium bicarbonate	Sodium bromide	Sodium carbonate	Sodium chloride
Sodium citrate	Sodium diatrizoate	Sodium dichromate	Sodium ferrocyanide
Sodium hydroxide	Sodium molybdate	Sodium nitrate	Sodium PO <sub>4</sub> di-basic
Sodium PO <sub>4</sub> mono-basic	Sodium PO <sub>4</sub> tri-basic	Sodium sulfate	Sodium tartrate
Sodium thiocyanate	Sodium thiosulfate	Sodium tungstate	Strontium chloride
Sucrose	Sulfuric acid	Tartaric acid	Tetracaine hydrochloride
Trichloroacetic acid	Trifluoroethanol	Tris (hydroxymethyl)aminomethane	
Urea	Zinc sulfate		