Procedure for Freezing Proteins

Version Number: 5

Date: 06.16.04; 02.06.06
Author: R.Pufan
Edited by: R. Kim
Reviewed by: J. Jancarik, R. Kim, I.Ankoudinova

Materials/Reagents/Equipment	Vendor
0.5 and 1.5 ml Eppendorf tubes	
Purified target ready for setting up	
Liquid Nitrogen	

Procedure:

Freezing Procedure for proteins: Use 250 µl of protein to set up on Hydra and for immediate improvements. Prepare to freeze the rest of the protein as soon as it is received. **First, test freeze a 30 µl aliquot in a 0.5 ml microfuge tube by dropping it in liquid nitrogen, then store at -80°C for at least 1 hr. Thaw out, observe whether the protein precipitates**. If it does not precipitate, aliquot **100 µl** (if the amounts are really high you may also aliquot 300 µl) of protein in **0.5 ml** microfuge tubes. Label the protein gene ID # on the lid of the tube. Make sure you close them well and drop them in liquid nitrogen for a few seconds until frozen. Set all the microfuge tubes in a 50 ml conical tube. Label the tube with all information: **full protein name (vector and gene ID#), set, concentration, buffer, date and initials of purifier.** Set in designated area in –80°C freezer. Keep a log of # of tubes x 100 (or 300) µl = total volume of protein stored.. If the protein precipitates you may want to test freezing again by adding 5%-40% glycerol to your protein and proceed as above. When using the protein, dialyze or exchange the buffer to get rid of the glycerol.

Freezing procedure for work stopped or solved proteins: If a new preparation is made for a protein that is not crystallizing, or the protein is stopped or solved, the left over is frozen as well. **Make sure it is labeled the proper way: blue sticker for native, white for Se-met, full protein name (vector and gene ID#), set, concentration, buffer, date and initials of purifier**. Make sure you enter this information as well as the volume, and freezing date in the archive log. Drop the tube in liquid nitrogen and then set it in the plastic square box in the -80°C freezer .