Incorporation of cholesteryl hemisuccinate into detergent stock solutions

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Version 1.0

Summary/Abstract:

It is often useful to incorporate cholesterol or its analogs into detergent micelles to facilitate stability of solubilized membrane proteins. In order to efficiently include cholesterol in membrane protein purification, we have developed a protocol to solubilize 2% w/v cholesteryl hemisuccinate in 10% w/v detergent stock solutions. The protocol consists of two main stages: solubilization of dry detergent in 50mL of a 200mM Tris buffer pH 8; solubilization of cholesteryl hemisuccinate (CHS) in the detergent stock solution by sonication and mixing. After the above protocol has been carried out the solution will consist of a homogenous distribution of CHS in detergent micelles that should appear transparent. The protocol should take 30 minutes

Many membrane proteins rely on specific lipid interactions to retain function in the plasma membrane and stability in the detergent solubilized state. The G protein-coupled receptors in general have a requirement for cholesterol to retain folded active protein during the course of a purification. Efficient incorporation of cholesterol or cholesterol analogs into detergent micelles at a high enough concentration to be useful in purification protocols is often not straightforward due to the ability of different detergents to solubilize cholesterol.

Materials:

1. **Reagents:**

   n-dodecyl-B-d-maltopyranoside (Anatrace D310)

   1M Tris buffer stock pH 8.0 (Amresco, or any supplier)

   Cholesteryl hemisuccinate (Sigma C6512)

2. **Equipment:**

   Probe sonicator

   50mL falcon tubes

   Rotator

3. **Reagent Setup:**

   Weigh out 1g of cholesteryl hemisuccinate

Weigh out 5g of DDM

4. **Equipment setup:**

   Attach large probe tip to sonicator

   Set to 100% with no pulse

Step by step methodology

1 Add 30 mL of water to a 50mL falcon tube

2 Add 10 mL of 1M Tris pH 8 stock to above for a 200mM final buffer concentration

3 Add 5g of dry detergent to the vessel and screw cap on tight.

4 Invert tube or use rotator until detergent goes into solution

5 Add 1g of CHS to detergent solution.

6 Sonicate continuously until solution is hot to the touch and becomes translucent

7 Bring volume to 50mL with water

8 Place tube on rotator at room temperature until the solution becomes transparent

9 Cool to 4^{o}C on ice

References

1. Hanson MA, Cherezov V, Griffith MT, Roth CB, Jaakola VP, Chien EY, Velasquez J, Kuhn P, Stevens RC.. “A specific cholesterol binding site is established by the 2.8 A structure of the human beta2-adrenergic receptor.”, Structure. 2008Jun;**16**:897-905.

Please send comments, suggestions, and/or questions to Professor Ray Stevens (stevens@scripps.edu)