

Large Scale Preparation of Membrane Containing Over-expressed Proteins from SF9 Cells
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Version 1.0

Summary:

This protocol is currently being used at the JCIMPT to prepare samples of membranes containing over-expressed proteins from biomass of SF9 cells. The protocol is carried out to prepare a membrane homogenate prior to membrane extraction and detergent solubilization. This step remains to be the most time consuming and manual step in the membrane protein structural biology purification process and new JCIMPT processes including a newly designed instrument will be replacing this protocol.



Figure 1 Dounce Homogenizer used in the protocol

The protocol has three major steps, homogenization, washing with high salt, and finally preparation for freezing and storage. This protocol is used in scale up studies of between 1-10 Liters of biomass. The current process takes up to 12 hours to complete using 5 L of biomass. The final product is a membrane preparation that will be used in extraction, solubilization, and purification of over-expressed proteins.

Overview of Steps:

1. Lysis by Dounce Homogenization
2. Spin down membranes (100k x g)
3. Wash membranes with hypotonic buffer and centrifuge repeat until clean (by commassie assay)
4. Wash membranes with 1M NaCl buffer and centrifuge repeat until clean (by commassie assay)
5. Store in 40% glycerol Buffer at -80C

Materials:

1. Reagents:

Lysis Buffer: (10 mM HEPES pH7.5, 10 mM MgCl₂, 20 mM KCl)

Bradford Assay reagent (Pierce)

Please note, depending on the susceptibility to proteolyses protease inhibitors may have to be added throughout the membrane preparation process. At JCIMPT, we have been using the following inhibitors when working with GPCR proteins:



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1. Protease Inhibitor Cocktail Set V (calbiochem)
2. Bestatin (Roche)
3. Leupeptin (Roche)
4. Pefabloc (Roche)

2. Equipment:

Dounce Homogenizer
Centrifuge

Step by step methodology:

Preparation from 1-10 Liter biomass have been used with this protocol, here we present the details when working with 5 L of biomass, please adjust accordingly for other volumes.

Homogenization

- a. Suspend 5L of cell culture in 400mL of lysis buffer
- b. Centrifuge cells in Ti45 tubes (70mL) at 45000rpm for 45 minutes
- c. Pour off supernatant
 - i. Test for protein using Bradford assay
- d. Resuspend cell pellets in 100mL chilled lysis buffer
 - i. Homogenize with Dounce
 1. Break up pellets
 2. 20 additional strokes
- e. Dilute membranes to 400mL in lysis buffer
- f. Repeat steps c-e three times

High salt wash

- g. Add NaCl to lysis buffer final concentration 1M
- h. Repeat steps c-e in High salt buffer as necessary until no protein is detected in Bradford assay using 10uL protein 100uL assay reagent

Freezing and Storage

- i. Dilute membranes to final concentration of 2g/mL
 - i. based on initial mass of cell pellet
 - ii. add 40% final conc. Glycerol to lysis buffer with no additional NaCl
- j. Drop 10mL of suspension into liquid N₂ filled Falcon tube for flash freeze (**WARNING:** wear appropriate eye protection at this step)
 - i. Maintain at liquid N₂ until transfer to -80°C for long term storage

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