Kamat Lab Thin Film Hydration Protocol

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Abstract

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Protocol

Assemble materials

Step 1.

Materials:

Glass vials (any will do, but we use 2mL Fisherbrand Class B Clear Glass Threaded vials: cat #: 03-339-21A)

Glass syringes of various sizes (ex. Hamilton gastight cat #: 14-815-238, but any will do) for use with lipids and chloroform

Phosphate buffered saline 290 mOsm (Sigma, P4417-100TAB)

Mini Extruder (Avanti Polar Lipids)

Polycarbonate Membranes (Whatman Nuclepore Track-Etched Membranes, 19 mm)

NOTES

This is a general procedure to prepare phospholipid films with and without a Lissamine Rhodamine Membrane Dye to be hydrated later to prepare lipid vesicles. This procedure works best for studies with bulk small unilamellar vesicles.

Final working conditions

Step 2.

Final working conditions

Reagent	Source	Final working concentration
DOPC	Avanti 850375C	2.5 E -5 mols/vial (1 mL of hydration yields 20 mM vesicles)
Rhodamine Lissamine PE	Avanti 810150 C	0.1 mol % of POPC

Prepare lipid films in glass vials

Step 3.

1. Determine the volume and molarity DOPC vesicles you want to create (ex: 1 mL of 20 mM DOPC vesicles = 2.5E-5 mols of DOPC. The stock of DOPC is 25 mg/mL. To make giant, nice looking vesicles, it's best to prepare a more dilute sample of vesicles (like 200 uM). For small unilamellar vesicles, you can work with much higher concentrations of lipids (we can go up to 130 mM for DOPC))
2. Add 2E-5 mols DOPC and appropriate amount of Rhodamine PE into a glass vial since you are working with organic solvents. Cap and vortex briefly to ensure the two components are well mixed.
3. Remove cap and allow the chloroform to evaporate away in the hood.
4. Place the vial into a vacuum chamber for > 1 hrs or leave overnight in order to remove all residual traces of solvent.
5. Cap and store vials in freezer until ready to be used and hydrate with 1 mL of aqueous hydration buffer when you are ready to prepare vesicles.

Hydrate films and extrude vesicles

Step 4.

- Add 1 mL of PBS to each vial, place in 60°C oven for 1 hr, then vortex 10 seconds. Lipids should easily assemble. If clumps or aggregates persist after several vortexing attempts, this is often a sign something went wrong in vesicle assembly. The heating step is not necessary and can be excluded, but helps when other membrane components are involved like diblock copolymers.
- Extrude films at room temperature through 100 nm polycarbonate membranes using a mini extruder and heating block described on the Avanti website (7 passes through membrane is fine, 9-11 is better, more than that is unnecessary according to my post extrusion analysis of vesicle size distribution on DLS.)

Purify vesicles if hydration buffer contained a dye or solute (ex. calcein)

Step 5.

Set up columns (BioRad Poly Prep 7311550) containing 6 mLs of Sepharose 4B (size exclusion column media). Purification should be conducted on the same day as a subsequent assay.