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Morrissey Lab Protocol for Preparing Phospholipid Vesicles by Extrusion

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http://tf7.org/mlabprotocols.htm

Background:

In this method, phospholipids are first suspended in a buffered saline solution to give large, multilamellar vesicles. The vesicles are then repeatedly passed through a polycarbonate filter with 100 nm pores. The result is uniformly sized, unilamellar vesicles (large unilamellar vesicles, or LUV), approximately 100 nm in diameter. We use the " Liposofast " extruder from Avestin, Inc. (2450 Don Reid Drive, Ottawa, Ontario, Canada K1H 1E1; Tel: 613-736-0019). It is a syringe-based membrane extruder that is inexpensive and easy to use, and it allows one to prepare 0.5 to 1 ml batches of phospholipids at a time. Avanti also sells a vesicle extruder, which they call the Mini-Extruder.

Important note: The phospholipids must be handled at a temperature above their transition temperature (T_c) from gel to liquid crystalline phase. We generally use natural phospholipids, which are in the liquid crystal phase at room temperature. If you use other types of phospholipids you will need to carry out all these procedures at a temperature above the T_c, not necessarily at room temperature!

We use these vesicles in our research on blood clotting. Alternative procedures to make unilamellar vesicles include sonication, detergent/dialysis and detergent/Bio-Beads.

Method:

1. Dispense 2.6 µmole total phospholipids (PL) in a *glass* test tube (a 13 x 100 mm tube is a convenient size).

***Examples**—amounts of PL to use in making PCPS or PCPSPE vesicles:*

For PC:PS vesicles (80:20 molar ratio)		
63 µ**L PC** (at 25 mg/ml) (or **158** µ**L** at 10 mg/ml)	= 1.58 mg	= 2.08 µmole
42 µ**L PS** (at 10 mg/ml)	= 0.42 mg	= 0.52 µmole

For PC:PE:PS vesicles (40:40:20 molar ratio)		
32 µ**L PC** (at 25 mg/ml) (or **79** µ**L** at 10 mg/ml)	= 0.79 mg	= 1.04 µmole
80 µ**L PE** (at 10 mg/ml)	= 0.80 mg	= 1.04 µmole
42 µ**L PS** (at 10 mg/ml)	= 0.42 mg	= 0.52 µmole

Be sure to overlay the contents of the stock vials of phospholipid with argon gas before capping and returning them to the freezer!

2. In the fume hood, dry the PL mixture under a gentle stream of nitrogen or argon. When dry, speed-vac for an additional 1 hr to overnight under high vacuum. (This is to remove any residual chloroform.)

3. To the dried-down PL, add 2.6 ml room temperature HBS solution and cover the end of the tube with parafilm. Incubate 1 hr at room temperature with intermittent agitation.

4. Vortex tube vigorously to completely resuspend the PL. The result should be a milky, uniform suspension. Freeze and thaw the suspension three to five times. (Freeze in dry ice/alcohol bath; thaw rapidly at 37°C.)

5. Clean the Liposofast device with ethanol and dry it well. Assemble the device with two membranes held between the two "O" rings and filter supports according to the manufacturer's directions. (We generally use two polycarbonate membranes with 100 nm pore size, although other pore sizes can also be used.)

6. Load 0.5 ml of the lipid suspension into one of the two glass syringes and attach it to the Luer lock on one side of the device. Close the other (empty) syringe and attach it to the Luer lock on the opposite side of the device.

7. Press the loaded syringe to pass its entire contents through the filter and into the opposing syringe. Repeat this process alternately with the two syringes for a total of at least 11 passes. *It is essential that you always use an odd number of passes, so that the final product will end up in what was originally the empty syringe. This will ensure that none of the starting multilamellar vesicles will contaminate the final product. In addition, it is critical that this procedure be performed at a temperature that is above the* T_c *for your lipid mixture.*

8. Remove the final product and repeat steps 6 and 7 for the remaining, unprocessed phospholipid suspension, until all of the suspension has been processed.

9. Store the final product at 4°C. The result is a uniform suspension of unilamellar vesicles (about 100 nm in diameter) containing a total of 1 mM phospholipid in HBS.

Note:

You can confirm the final phospholipid concentration by assaying total phosphorus content .

Materials and Solutions:

HBS (store at room temperature)
100 mM NaCl 20 mM Hepes/NaOH buffer, pH 7.5 0.02% (w/v) sodium azide

(50 mM Tris buffer, pH 7.5 may be substituted for the Hepes buffer)

Phospholipid Stock Solutions*			
	Phospholipid name	*concentration*	*MW*
PC	L-alpha-Phosphatidylcholine, egg	10 or 25 mg/ml	761
PS	L-alpha-Phosphatidylserine, bovine liver-Na salt	10 mg/ml	810
PE	L-alpha-Phosphatidylethanolamine, bovine liver	10 mg/ml	768

*We buy our phospholipids from Avanti Polar Lipids , dissolved in chloroform. The stock solutions should be stored at -20°C under argon. Do not store more than 3 months (6 months for PC).

References

Mui B Chow L & Hope MJ (2003) Extrusion technique to generate liposomes of defined size. *Methods Enzymol* **367:**3-14.

http://www.avantilipids.com/extruder.html

http://www.avantilipids.com/ExtruderAssembly.html

http://www.avantilipids.com/LUVET.html

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