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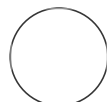
🌐 Preparation of unilamellar liposomes

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Protocol status: Working
We use this protocol and it's working

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ABSTRACT

We present here a protocol for preparing liposomes that can be used to monitor binding of proteins to vesicles of various membrane curvatures. We used this to study the association of the PPM1H phosphatase with highly curved membranes due to its N-terminal amphipathic helix.

PROTOCOL integer ID:
87516

Keywords: ASAPCRN

- 1.(18:0-20:4)PC (1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphocholine)
[Avanti #850469]
- 2.(18:0-20:4)PI (1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphoinositol)
[Avanti #850144]
- 3.(18:0-18:2)PS (1-stearoyl-2-linoleoyl-sn-glycero-3-phospho-L-serine)
[Avanti #840063]
- 4.(18:1)PI(4)P {1,2-dioleoyl-sn-glycero-3-phospho-(1'-myo-inositol-4'-phosphate)}
[Avanti #850151]
5. Cholesterol sulfate [Avanti #700016]
6. 0.4 µm pore size polycarbonate filters [Avanti #610007]
7. 0.1 µm pore size polycarbonate filters [Avanti #610005]
8. 0.05 µm pore size polycarbonate filters [Avanti #610003]
- 9.Chloroform [Fischer Chemical #C298-500]
- 10.Hand held mini extruder with heating block [Avanti # 610000]

Make lipid stocks

- 1 All lipids were purchased from Avanti Polar Lipids. PC and PS were obtained as 10mg/ml chloroform solutions. PI, PI(4)P and cholesterol sulfate were obtained as powders. Dissolve in

chloroform to prepare 2mg/ml, 1mg/ml and 25mg/ml stock solutions respectively.

Prepare the lipid mixture

2 Prepare unilamellar vesicles by mixing:

🧴 940 μL of (18:0-20:4)PC

🧴 470 μL of (18:0-20:4)PI

🧴 60 μL of (18:0-18:2)PS

🧴 140 μL of (18:1)PI(4)P

🧴 26 μL of cholesterol sulfate

from the respective stock solutions in a glass vial. The lipid composition of the liposomes is in the ratio (mol %) 78:7:5:1:9 to represent the mammalian cell Golgi composition (Fasimoye et al., 2023).

3 Dry the above-mentioned lipid mixture in chloroform under nitrogen flow by pointing a Pasteur pipette into the glass vial. This vial is subsequently incubated under house vacuum for at least

🕒 01:00:00

1h

4 Resuspend the dried lipids by pipetting up and down in 1ml of resuspension buffer (50mM HEPES pH 7.5, 120mM KCl)

Liposome preparation

5 Sonicate the liposome suspension by two brief 🕒 00:00:05 cycles of bath sonication followed by sequential extrusion through 0.4, 0.1 and 0.05 μm pore size polycarbonate filters for 21 times using a hand extruder.

5s

Liposome storage

6 The final lipid concentration of the liposome suspension will be 15mM; store at 🌡 4 °C for a maximum of 2 weeks. Small liposomes are best used close to the time of their preparation.

