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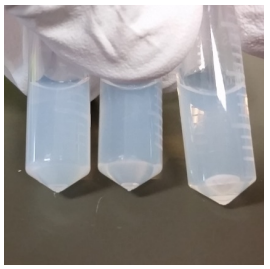
General preparation of liposomes using probe-tip sonication



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Monica Rieth[1], Andrew Lozano[1], Jordan Grant[1]

[1]Southern Illinois University-Edwardsville

Labyrieth

Monica Rieth
Southern Illinois University-Edwardsville

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

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Abstract

This method outlines a general approach for preparing liposomes using probe-tip sonication. The method has been optimized for the preparation of pure DPPC liposomes on a 25-mg scale and may require modifications as the quantity of lipid is altered or upon addition of other lipids and small molecules.

Materials

1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) lipids can be purchased commercially from Avanti Lipids either as a dried powder or dissolved in chloroform.

The lipid powder can be dissolved in ethanol or hexane as an alternative to chloroform.

Safety warnings

Work in the hood if you are using lipid dissolved in chloroform.
Ear protection should be used with a sonicating apparatus.

Before start

Allow lipids to reach room temperature prior to weighing.

1 Lipids are typically stored at -20 °C and should be allowed to reach room temperature prior to working with them.
For DPPC powder, carefully weigh out 25 mg on a clean analytical balance using clean, ungloved hands (to minimize static)

2 Carefully transfer the lipid to a clean 2.0 mL glass vial. Add 2.0 mL of (0.2 um-filtered) buffer. (20 mM HEPES, 100 mM NaCl, pH 7.4)

3 Samples were vortexed to mix and hydrate the lipid powder or dried film (if prepared from a chloroform solution lipids should be dried to a film under a stream of nitrogen gas).

4 To suspend the lipids more homogeneously and remove large particulates the mixture can be sonicated using a probe-tip sonicator (Fisher Scientific, Hampton, NH) set to 20% duty cycle with a pulse time of 2 seconds followed by a rest period of 2 seconds for a **total sonication time of 2 minutes**.

5 To prepare liposomes from this mixture, the cycle in step 4 should be repeated 3 additional times for a total of 4 cycles at 2 minutes total sonication time per cycle. Total liposome preparation time is 8 minutes.

Note

The rest period is important to avoid excessive heating of the sample. Temperature should always be monitored closely.

6 Samples were centrifuged using a standard benchtop microcentrifuge at 10000 x g for 3 minutes to remove residual titanium particles from the sonicator probe tip and un-reconstituted lipids.

7 Carefully remove the supernatant and transfer to a clean 2.0 mL Eppendorf tube.

8 Samples can be stored at 4 °C for up to 24 hours. An additional centrifugation step should be carried out on samples that have been stored to remove any precipitated lipid.