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 We use this protocol and it's working

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General preparation of liposomes using probe-tip sonication

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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 INSTRUCTIONS

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.