Protocol Small Unilamellar Vesicles (SUVs) formation

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Materials:

1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC)
HBS solutions (20mM Tris pH 7 +150mM NaCl)
Round bottom glass test tube
Glass pipettes and vials
Probe tip sonicator
Centrifuge
Vacuum desiccator

Protocol:

1) Dissolve DMPC lipid in chloroform to a concentration of 20ug/mL.
   DMPC is ordered from Avanti in 50ug containers. Chloroform should be added directly into the container and then several aliquot can be made in small glass vials. **Use glass pipettes and containers**; plastic reacts with chloroform. Be sure to work quickly to avoid oxidation of the lipids. Container caps should be sealed securely and rapped in parafilm.

2) Add 100uL of the Chloroform/lipid mix to a **clean** round bottomed glass test tube.

3) Evaporate this solution under a stream of nitrogen until dry. A thin film of lipids should be visible at the bottom of the test tube.

4) Place the test tube in a vacuum desiccator for at least 90 minutes to remove any residual chloroform.

5) Pre-heat 1mL of HBS solution to above 23 Centigrade (it is important that the lipids hydrate above their transition temperature of 23 C)

6) After removal from the desiccator, add HBS solution to the test tube. Cover with parafilm and incubate in a water bath over 23 degrees Centigrade for 1 hour. (Note, if room temperature is above 23C it does not need to be placed in a bath.)

6) Remove test tube from the bath and vortex briefly until the solution appears milky.

7) Sonicate the lipid/HBS solution with the probe tip sonicator.
   Place the test tube in a holder and surround with ice to prevent overheating.
   The tip of the sonicator should be placed just below the surface of the liquid, with care taken so that it does not touch the sides of the glass.
   Use a sonication protocol in which the tip actively sonicates for 59 seconds and rests for 30 seconds, with a total run time of at least 15 minutes (more if liquid has not cleared.)
   If the sonication is successful, the solution will clear significantly, becoming nearly transparent (slight cloudiness will remain). If this does not occur, continue sonication.

8) The now clear solution should be spun down in a centrifuge to remove any titanium from the sonicator probe and remaining non-unilamellar vesicles.

Spinning at 16,200 rcf for 20min is sufficient.

9) Remove soup from centrifuge and place in a glass vial. Store SUVs at 4 degrees Centigrade and use within 24 hours.

Other lipids can be used. See Avanti's website for more details and a list of transition temperatures suitable lipids. http://www.avantilipids.com/LipidsForLiposomeFormation.html