Lipid Preparation Protocol

1) Stock lipids are dissolved in organics such as Chloroform.

DOPC (25 mg/ml or 32 mM) - 1 ml = 25 mg or 32 umol

DOPG (25 mg/ml or 31 mM) - 1 ml = 25 mg or 31 umol

DOPS (10 mg/ml or 12.3 mM) - 1 ml = 10 mg or 12.3 umol

Brain PIP2 (1mg/ml or 0.9 mM)

2) Mix ~25mg (1ml) of lipids in a glass tube (~ 25mg lipid in 1 ml/tube)

a) 670 ul **DOPC** (67 mol% = 21.4 umol) + 330 ul **DOPG** (33 mol % = 10.2 umol) = (100 mol % = 32 umol)

b) 700 ul **DOPC** (70 mol % = 22.4 umol) + 752 ul **DOPS** (29 mol% = 9.2 umol) + 351 ul **Brain PIP2** (1 mol% = 0.32 umol) = (100 mol % = 32 umol)

c) 1ml **DOPC** Alone (100 mol % = 32 umol)

d) 1ml **E.coli Phospholipid Extract** (25 ml ~ 32 umol)

* To visualize the Small Unilamellar Vesicles (SUVs), add 0.5 mol % of Rhodamine-PE (MW=1320, Ex560nm/Em583nm):

1) Re-suspend 5mg of powder with 5ml CHCl3 (cf = 1mg/ml or 758uM).
2) Add 211ul to achieve 0.5 mol % to the lipid mixes above.

3) Split volumes of each mixture into 2 glass tubes (16 umol/tube)

4) In fume hood, use dry N_2 gas to dry lipid with rotation to make a thin film of dry lipid cake.

5) Speed vacuum to dry completely for 2hr @ 42^{o}C for 1st hour.

6) Add 2.5ml degassed TK150 buffer to each tube in N_2 box (C_f ~ 5mg/ml or 6.4 mM) and seal with parafilm.

7) Vortex for 1min, spin down, and hydrate o/n at RT in dark. Next day vortex for 1 min. and spin down.

8) Sonicate with Qsonica model #Q700A with #431C2 Cup Horn, #440 tube rack and #4900 Chiller.

- Use of a chiller is highly recommended to accurately control temperature.
- Maintain water level inside Cup just above the sample in each tube.
- Use 1.5ml Polystyrene tubes (standard polypropylene tubes do not work efficiently).
- Sonicate at 15% amplitude setting (70 watts) using pulse mode to help control temperature.
- Sonicate 0.5ml/tube until translucent.

Sonication times:

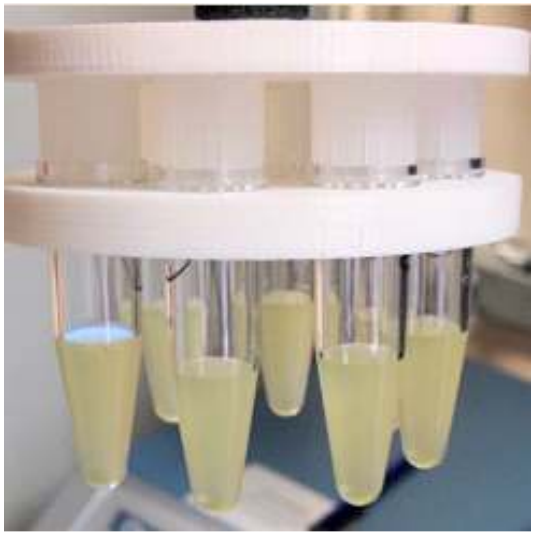
- 2 min (30s on, 10s off) for E.coli phospholipid extract
- 5 min for 33% DOPE + 67% DOPC
- Longer time period needed for DOPC alone (may need higher amplitude/wattage – 80W)

9) Spin down and syringe filter SUVs.

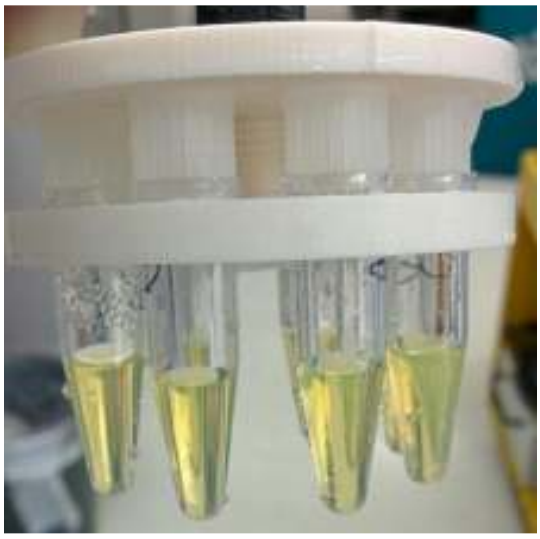
10) Make ~ 200ul aliquots, layer with N_2 gas, and store @ 4°C Teflon capped and parafilm sealed tubes.

* Aliquots are diluted to 0.5mg/ml with TK150 buffer and 5mM MgCl2 before flowed into flowcell to make flat supported lipid bilayers.
* Lipid stocks are good for ~1 month after which they get cloudy as vesicles fuse over time.

TK150 Buffer	**[STOCK]**	**[1x]**	**1x (10ml)**	**[2x]**	**2x (30ml)**
Tris-HCl (pH 7.4)	1 M	25 mM	0.25 ml	50 mM	1.5 ml
KCl	3 M	150 mM	0.5 ml	300 mM	3.0 ml
H_2O			9.25 ml		25.5 ml



Before Sonication



After Sonication