Preparing Lipids

Components, from Avanti Polar Lipids:

DOPC: 1,2-dioleoyl-sn-glycero-3-phosphocholine, as 1g powder

PEGylated lipids: 18:1 PEG2000 PE

1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (ammonium salt), as 100mg powder

biotinylated lipids: 18:1 Biotinyl Cap PE

1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(cap biotinyl) (sodium salt), as 25mg powder

Prepare the Master Mix (MM) in the DOPC glass bottle. The final proportions are:

100mg/ml DOPC

10mg/ml PEG-2000 DOPE

.5mg/ml biotinylated DOPE

Use up the entire 1000mg of the DOPC, so the final volume of chloroform will be 10ml. This translates to 100mg total PEG-2000 DOPE and 5mg biotinylated DOPE.

1) Take out all components from the freezer and leave at room temperature for 30 minutes to 1 hour. This will prevent water condensation when the vials are opened.

2) Thoroughly clean a 10ml glass pipette.

3) Open the 1g DOPC bottle and add 100mg PEG-2000 DOPE directly into it. The PEG-2000 DOPE is, conveniently, available as a 100mg vial.

4) Then measure out and add ~5mg biotinylated PEG.

5) Rinse the glass pipette with chloroform. Dissolve the dry lipid mix in 10ml chloroform. Mix gently by swirling until all the dry lipid is dissolved.

6) Seal the bottle with plenty of parafilm and store at -20.

1) **To prepare lipids for experiments**, rinse a small glass vial with water then alcohol; dry it with nitrogen, and place it in the vacuum oven for 30 minutes or so.

2) Take the MM out of the freezer and let it come to room temperature to prevent condensation on opening.

3) Rinse a 1ml (or similar) Hamilton syringe (with a metal plunger!) with chloroform. Then meaure out 200ul of the MM and transfer it to the cleaned glass vial.

4) Slowly evaporate the chloroform with a slow nitrogen stream. Try to get the lipids to dry in a film across the side of the vial, up to a height of a few cm. Make sure all the chloroform, including pockets caught under the lipid film, is evaporated. You can up the nitrogen pressure as the chloroform evaporates.

5) Place the vial under vacuum overnight. It can stay like this for several days if necessary.

6) Resuspend the lipids in 2ml lipid buffer at room temperature for at least an hour. It can go overnight.

7) Vortex and sonicate. Ask someone about sonication procedures for lipids–everyone has a different approach.