

Protocol for Generation of lipid stock for LCP (7-19-15)

Monoolein (9.9 MAG) is purchased from Hampton Research (Cat. no. HR2-435) and derivatives from Avanti Lipids (*i.e.*, 7.7, 9.8, and 9.7). Typically 1 g or 100 mg of Monoolein or its derivatives are reconstituted, respectively.

- 1) Remove lipids and cholesterol (Sigma, C8667) from freezer; make sure to allow the bottles and their entire contents to equilibrate to room temperature before opening. The presence of water will make it difficult to transfer lipid.
- 2) Transfer desired amount of lipid into 24mL **glass** screw cap vials (Kimble Chase, cat. no. 60940A) using micro-balance to accurately determine the **EXACT** weight of lipid.
 - a. Monoolein is in wax form and can be directly transferred to glass vial by scraping it out with a spatula, record exact weight of transferred material.
 - b. Derivatives from Avanti typically come as oils, transfer commercial 100 mg stock plastic vials to 50 mL conical tubes and pellet oil at 1000 x RPM for ~1 min. If contents are still not pelleting, heat vials to ~ 42 °C for 5-10 min and then repeat spin step. Transfer contents into glass vial using a P200 pipette; make sure to cut off tip since the solution is very viscous. For quantitative transfer, stock vials may need to be centrifuged again. Remember to record exact weight of transferred lipid.
- 3) In a 4 mL glass crew cap vial (Kimble Chase, Cat# 60940-A), weigh out 10% of the lipid weight determined in step 2. Be as accurate as possible.
- 4) Resuspend the cholesterol under a chemical hood with ~1 mL of chloroform using a glass Pasteur pipette. Once majority of cholesterol is resuspended, gently wash sides to collect residual cholesterol stuck to sides of vial.
- 5) Quantitatively transfer reconstituted cholesterol into 24mL vial containing lipid
 - a. Carefully transfer reconstituted cholesterol into lipid vial, set aside pipette for 2nd transfer, see below. Do not discard since there will likely be residual amounts of lipid or cholesterol on pipette tip.
 - b. Using a new Pasteur pipette, add another ~1 mL of chloroform to 4mL vial containing residual cholesterol. Use the used pipette in step 5a to transfer this chloroform wash to the lipid vial. It is very important not to contaminate chloroform stock and to get quantitative transfer of cholesterol into lipid.
 - c. Repeat step 5b if there is any doubt of quantitative transfer
- 6) Dry chloroform off with a steady stream of argon gas in chemical hood. Use purification stand with 2 clamps, make sure lipid stock is slightly tilted and not straight up and down. Attach a glass pasteur pipette at the end of the argon tubing, insert ~¼ down glass lipid vial. Gently turn on Argon until the chloroform lipid mixture is agitated enough where the solution is "rumbling". Too little flow will create a lipid cap and prevent complete chloroform removal.
- 7) Dry overnight in vacuum, make sure to cover with foil
- 8) Aliquot lipid accordingly, 9.9 will freeze at RT and thus may need to be warmed up to 37-42 °C prior to aliquoting.
- 9) Overlay aliquots with argon and store at -80 °C.