Selected Laboratory Protocols: Preparation of ^{14}C-Oleate-Albumin Solution for Whole-Cell Cholesterol Esterification Assay

1. 24% (w/v) BSA

In a 50-ml beaker with a stirring bar, add 17.5 ml 150 mM NaCl. Slowly dissolve 6.25 g fatty acid-free BSA (Sigma) by adding 2 g every 50 min while stirring at room temperature. Adjust pH to 7.4 with 5 N NaOH. Finally, adjust to 25 ml with 150 mM NaCl. Store at –20^{0}C.

2. 12.7 mM Na-oleate-albumin

Weight out 90 mg oleate (Sigma) by pipetting the oil into a microfuge tube that has been placed in a scintillation vial on a scale. Then transfer the oleate to a 50-ml beaker by using multiple 100-µl absolute EtOH wash-transfers. Add EtOH so that the final volume of EtOH is 2 ml.

Add 100 µL 5N NaOH and mix. Cover the beaker with aluminum foil and poke small holes in the top. Remove EtOH by evaporation under a gentle stream of N_2 until completely dry—usually takes 1-3 hours. Carefully disrupt the soapy paste with a spatula to enable evaporation of EtOH that is trapped in the paste. NB: the aluminum foil prevents the dried soap from blowing away.

Prepare a 60^{o}C water bath by placing a shallow pool of water in a large beaker on a heating plate and adjusting the temperature until the water is a steady 60^{o}C. Add 10 ml of 150 mM NaCl to the dried oleate soap, gas with argon, cover tightly with aluminum foil, and place in the 60^{o}C water bath. Stir while heating until clear (10-20 min at 60^{0}C). Then stir at room temperature to cool slightly.

While still warm, rapidly add 13 ml of ice-cold 24% BSA, gas with argon, cover with parafilm, and stir for a few minutes. Adjust to 25cc with 150mM NaCl, gas with argon, cover, and stir for few minutes. Finally, store under argon at -20^{0}C. Note that the argon is important because oleate is easily oxidized.

NB: The procedure calls for 12.7 mM Na-oleate and 1.79 mM albumin (ratio 7.1 :1), which is close to threshold. Therefore, if the albumin solution is less than 24% (*e.g.*, due to the loss of some BSA powder during the preparation of the solution), precipitation on cooling of albumin-oleate will result. To be safe, we usually add an extra 0.3 mg FA-free BSA to the solution. Test clarity by placing in borosilicate tube and holding up to a light. In a similar vein, if the solution is cloudy on thawing, add 1cc 24% BSA and stir at 60^{0}C for 0.5 hour.

3. ^{14}C-oleate-albumin

Add 250 µCi ^{14}C-oleic acid to a 25-ml Erlenmeyer flask and dry under N_2. Add 4.35 ml of the Na-oleate-albumin solution prepared above. Then add 1.6 cc 12% BSA in 150 mM NaCl pH=7.4, which was prepared by diluting the 24% BSA solution. Gas with argon, seal with parafilm, and stir gently at room temperature for 4-6 h. Filter the solution using a 0.45-µm Millipore filter fitted on a syringe. Store in aliquots under argon at –20^{0}C. Final oleate concentration is 10 mM.