Labeling of Acetyl-LDL with DiI (1,1'-dioetadeeyl-3,3,3',3'-tetramethylindocarboeyanine perchlorate)

Day 1:

1. Incubate 2 mg acetyl-LDL (300 µl of a 6.7 mg/ml stock) with 100 µl of 3 mg/ml DiI (in DMSO; from Molecular Probes) + 4 ml lipoprotein-deficient serum

NB: DiI is light sensitive so store solution at 4°C protected from light. Powder is stored at-20°C.

2. Incubate in a sterile tube for 8 h at 37ºC.

3. Add 0.5 g of NaBr and mix well. Divide among two tubes for the 50.3 Ti rotor. Overlay with a NaBr solution of 1.063 g/ml.

4. Centrifuge at 35,000 rpm o/n.

5. To prepare for dialysis, make several liters of LP buffer (0.9 % saline/0.3 mM EDTA, pH 7.4) and place at 4°C.

Day 2:

1. Collect the top layer of DiI-acetyl-LDL using a fine capillary tip, and dialyze against 1-4 liters of LP buffer x 3 changes.

3. Collect sample (volume should be ~250 µl), which may need to be microfuged briefly to rid of particulate material. Usual protein concentration is 8 mg/ml.

4. example ((2mg/.24ml) =8mg/ml)

Incubations and microscopy:

1. Good labeling of macrophages is obtained using 10 µg/ml for 60 min.

2. After incubations, rinse monolayer with PBS and incubate with 2% paraformaldehyde in PBS for 10 min at RT.

3. Rinse cells 5 times with PBS, and view under microscope in PBS using the Cy3 channel.