OIL RED-O STAINING OF FAT CELLS (IN CULTURE)

Quan Kang, 10/4/05, Updated by Chris Song, 2/2/06, Commented by TCH

REAGENT PREPARATION:

1) Prepare **Oil Red-O stock**: Add 0.5gms of Oil Red-O powder (FisherBiotech) into 100 ml of isopropanol. Stir overnight in a glass bottle. Then filter the mixture through **two layer Whatman papers**.
2) Prepare a **fresh** Oil Red-O working solution by adding 6.0ml of stock to 4.0ml of DD water. Then filter the mixture through **two layer Whatman papers**. (**NOTE**: It's absolutely important to remove any precipitates).
3) Prepare fixative solution. (11.0ml of 37% formaldehyde + 29.0ml PBS; final concentration is 10%).

STAINING PROCEDURE:

1) Remove media completely, and rinse cell with PBS (**NOTE**: Rinse step may be skipped, especially if the cells are not healthy).
2) Aspirate completely, and add enough volume of fixative solution to cover cells and wait 20-30min at RT.
3) Gently wash the fixed cells 1-2 times with PBS.
4) Aspirate **COMPLETELY** (**NOTE**: ***It's VERY important to remove any remaining aqueous solution; or you will see a lot Oil Red-O precipitates***).
5) Add the freshly prepared Oil Red-O working solution, and incubate for 50-60 min at RT. (**NOTE**: You can leave your cells on a rocker).
6) Remove the staining solution and wash cells with PBS, 1-3 times.
7) Keep cells in PBS, and/or store the stained cells at 4°C.