Determination of Cellular Cholesterol Content

Reagents Needed:

Wako CII Cholesterol CII assay kit

Extraction Buffer: Hexane/Isopropanol (3:2)

Protein Solubilization Buffer: 10 mM sodium borate, 1% SDS

Phosphate-buffered Saline (PBS)

Protocol:

The protocol is designed for cells plated in 6-well dishes.

1. Wash cells twice with 1 ml cold PBS.
2. Add 2 ml of hexane/isopropanol to each well. Rock at room temperature on the Red Rocker for 1 hour to extract lipids
3. Transfer supernatants to glass tubes and remove solvents using the SpeedVac.
4. Add 1 ml of the Cholesterol CII (Wako) buffer solution and vortex to solubilized the lipid pellet
5. Incubate at 37˚ C for 10 min.
6. Read absorbance at 505 nm.

Note: Color development with cholesterol is stable for incubation periods of up to 60 min. at 37˚ C. If detecting ent-cholesterol, incubate samples at 37˚ C for 1 hour, as the reaction is slower for this isomer.

To determine cellular protein content:

1. Add 1 ml of protein solubilization buffer to the cell monolayer left behind after lipid extraction.
2. Incubate 5 min at room temperature
3. Using a pipette, wash the solubilization buffer over the monolayer
4. Transfer solution to tubes and vortex
5. Use ~10 μl aliquot for BCA protein assay.

Report cholesterol content on a μg cholesterol/μg protein basis.