ab133116 – Cholesterol Assay Kit (Cell-Based)

Instructions for Use

A simple fluorometric method to study mechanisms and biological factors that regulate cholesterol metabolism or movement within cells.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

ab133116 includes filipin III, fixative, and wash buffer in a ready to use format. It provides a simple fluorometric method to study mechanisms and biological factors that regulate cholesterol metabolism or movement within cells. A cholesterol trafficking inhibitor, U-18666A, is included as a positive control.

2. Background

Cholesterol is both an important structural component of cell membranes and an early intermediate in hormone and bile acid biosynthesis. Cholesterol is not uniformly distributed among cellular membranes, but rather there are structurally and kinetically distinct cholesterol rich and poor domains. Under normal conditions, as much as 80-90% of total cellular cholesterol is present at the plasma membrane, whereas very little cholesterol resides in the endoplasmic reticulum and inner mitochondrial membranes. Cholesterol that is acquired by internalization and lysosomal hydrolysis of plasma lipoproteins, such as low-density lipoprotein (LDL), or synthesized in the endoplasmic reticulum is rapidly transported to the plasma membrane and integrated into the plasma membrane lipid pool. Within cells, intracellular cholesterol may move to different compartments through vesicular or nonvesicular pathways such as those mediated by diffusible carrier proteins. Defects in these transport pathways can alter cellular cholesterol metabolism resulting in pathological states. The mechanism for the ensuing movement of cholesterol from intracellular sites to their ultimate cellular destination is an unresolved question of fundamental importance in the areas of cell biology and medicine. Thus, defining mechanisms of intracellular cholesterol transport and identifying the cellular factors involved are of great interest.

Filipin III is the predominant isomer of filipin, the collective name given to four isomeric polyene macrolides isolated from cultures of

S.filipinensis. Filipin has been widely used as a probe for sterol location in biological membranes. Interaction with cholesterol alters the filipin absorption and fluorescence spectra allowing visualization with a fluorescence microscope capable of excitation at 340-380 nm and emission at 385-470 nm. Filipin's ease of use makes it a convenient tool for the histochemical identification of unesterified cholesterol both in vitro and in vivo.

3. Components and Storage

Kit will arrive packaged as a +4°C kit. For best results, remove components and store as stated below.

Item	Quantity	Storage
Cell-Based Assay Fixative	2 vials	RT
Cholesterol Detection Wash Buffer	1 vial	RT
Cholesterol Detection Filipin III	1 vial	-20°C
Cholesterol Detection Assay Buffer	1 vial	RT
Cell-Based Assay U-18666A	1 vial	-20°C

Materials Needed But Not Supplied

- A 6-, 12-, 24-, or 96-well plate.
- A fluorescence microscope equipped with a UV filter set capable of excitation and emission wavelengths of 340-380 nm and 385-470 nm, respectively.

4. Pre-Assay Preparation

NOTE: Filipin III is light sensitive. Do not expose to direct intense light.

Preparing the Filipin III Stock Solution

Dissolve the whole vial of Cholesterol Detection Filipin III in 200 µl of 100% ethanol. We highly recommend that you make small aliquots and store them at -80°C. *NOTE: Filipin III is very unstable in solution and its activity decreases significantly with each use.*

5. Assay Protocol

A. Treatment of Cells

The following protocol is designed for a 96-well plate. Adjust volumes accordingly for other plate sizes.

1. Seed wells of a 96-well plate with 3 x 10^4cells/well. Grow cells overnight.

2. The next day, treat cells with experimental compounds or vehicle control for 48-72 hours, or for the period of time used in your typical experimental protocol. Cell-Based Assay U-18666A, a cholesterol transport inhibitor, is included in the kit to be used as a positive control (provided at a concentration of 2.5 mM). We recommend that you use serial of dilutions of U-18666A starting at 1.25 µM.

3. Examine cholesterol localization using the following staining procedure.

B. Histochemical Staining Procedure

NOTE: Perform all steps at room temperature.

1. Remove most of the culture medium from the wells.

2. Fix the cells with Cell-Based Assay Fixative Solution for 10 minutes.

3. Wash the cells with Cholesterol Detection Wash Buffer, three times, for five minutes each.

4. Dilute the Filipin III Stock Solution (prepared as described in Pre-Assay Preparation) 1:100 in Cholesterol Detection Assay Buffer. Add 100 µl of this Filipin III Solution to each well. Incubate in the dark for 30-60 minutes.

5. Wash the cells with wash buffer, two times, for five minutes each.

6. Examine the staining using a fluorescent microscope using an excitation of 340-380 nm and emission of 385-470 nm. Filipin fluorescent staining photobleaches very rapidly, thus the sample should be analyzed immediately.

6. Data Analysis

Performance Characteristics - Typical Staining Results

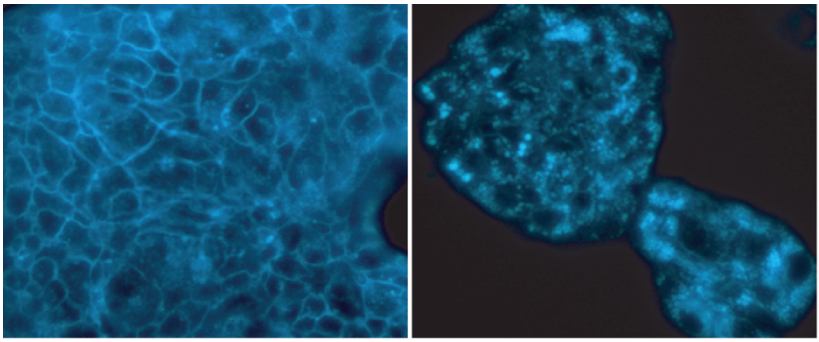

Figure 1. Accumulation of cholesterol inside HepG2 cells in response to 1.25 µM U-18666A. HepG2 cells were seeded in a 96-well plate at a density of 3 x 10^4 cells/well and cultured overnight. The next day, cells were treated with DMSO (vehicle) or 1.25 µM U-18666A for 48 hours. Panel A: Cells treated with DMSO alone demonstrate that majority of cholesterol is localized on the plasma membrane. Panel B: U-18666A treatment for 48 hours induces intracellular accumulation.

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