Cholesterol Detection Kit (cell-based) (BN00815)

(Catalog # BN00815; 100 assays; Store at -20°C)

I. Introduction:

Cholesterol is an essential structural component of animal cell membranes. It is required to maintain membrane structural integrity and fluidity. Cholesterol also serves as a precursor for the biosynthesis of several hormones, bile acid and vitamin D. Plasma membrane contains majority of the cellular cholesterol (80-90%) whereas little cholesterol resides in endoplasmic reticulum and in mitochondrial membrane. Transport of intracellular cholesterol within the cells to different compartments is through vesicular and non-vesicular pathways. Defects in these transport processes can alter cellular cholesterol metabolism resulting in pathological conditions. Filipin III is widely used as a probe for sterol localization in membranes. Interaction with cholesterol alters Filipin absorption and fluorescence spectra. This assay provides a simple, easy to perform, histochemical method of identification of unesterified cholesterol.

II. Application:

- Screen/study/characterize stimulators/inhibitors that affect cholesterol transport and localization

III. Sample Type:

- Cells

IV. Kit Contents:

Components	BN00815	Cap Code	Part Number
Fixative Solution	10 ml	WM	BN00815-1
Assay Buffer	100 ml	NM	BN00815-2
Staining Dye	10 µl	Red	BN00815-3
Inhibitor (U-18666A, 2.5 mM)	10 µl	Blue	BN00815-4

V. User Supplied Reagents & Equipment:

- 6-well, 12-well, 24-well or 96-well plate
- 100% Ethanol
- 37°C Incubator with 5% CO_2
- Light and fluorescence microscope with UV filter set Ex/Em = 340-380/385-470 nm.

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C. Read entire protocol before performing the assay. Open all reagents under sterile conditions (e.g. cell culture hood).

- **Fixative Solution:** Store at room temperature.
- **Assay Buffer:** Store at -20°C. Warm to 37°C before use.
- **Staining Dye:** Light sensitive, do not expose to intense light. Add 200 µl of 100% ethanol (not provided) to the vial. Aliquot and store at -80°C for long-term storage.
- **Inhibitor (U-18666A):** Store at -20°C. Dilute in Assay Buffer as per the assay requirement.

VII. Cholesterol Detection Protocol:

This protocol is for a 96-well plate. Adjust the volume according to the plate size.

1. **Cell Culture:** Seed 2-3 x 10^4 cells/well in a 96-well plate in desired media. Grow cells overnight in 37°C incubator containing 5% CO_2. Next day, treat cells with compounds of interest in 100 µl media. As a control, we recommend treating cells with vehicle alone. For inhibitor control, treat cells with diluted Inhibitor (U-18666A, a cholesterol transport inhibitor). Grow cells for 48-72 hrs or for desired time period.

   Note: Treatment with Inhibitor (U-18666A) will vary depending on the cell type. For HepG2 cell line we recommend using 1.25 µM.

2. **Cell Staining:** Remove culture media from wells. Wash cells with 100 µl Assay Buffer. Add 50-100 µl of Fixative Solution and incubate for 10 min. Wash cells with 100 µl Assay Buffer 2-3 times. Dilute Staining Dye 1:100 in Assay Buffer just before use and add 100 µl/well. Incubate in dark for 30-60 min at 37°C. Carefully remove the Assay Buffer containing dye using a pipette without disturbing the cells. Gently wash the cells 2-3 times with 100 µl Assay Buffer.

   Note: Cells can be stained directly with the Staining Dye without adding Fixative Solution (fixing of cells).

3. **Detection:** Examine cells using light and fluorescence microscope (Ex/Em = 340-380/385-470 nm). Acquire several images per well for analysis.

   Note: Since Staining Dye photobleaches very rapidly, we recommend analyzing samples immediately.

(a) (b) (c)

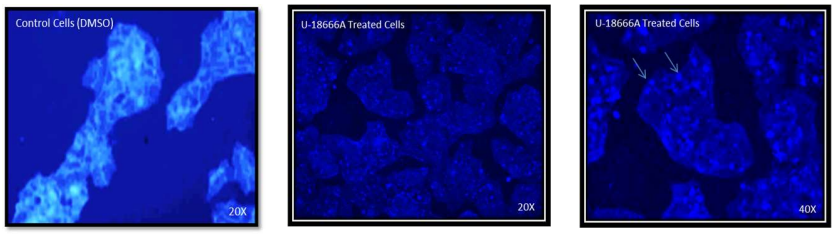

Figure: Liver cells (HepG2) cholesterol staining: HepG2 cells were cultured overnight and next day treated with U-18666A (1.25 uM) or vehicle control (DMSO) for 48 hrs. (a) Cells treated with DMSO alone demonstrated majority of the cholesterol is localized in Plasma membrane. (b) Treatment with U-18666A for 48 hrs resulted in intracellular accumulation of cholesterol. (c) Higher magnification of image B. Images were taken using Nikon TiE microscope.

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