

ab303783 – Cholesterol Detection Kit (cell-based)

For the screening of stimulators/inhibitors that affect cholesterol transport and localization.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab303783>

Storage and Stability

The entire kit may be stored at -20°C.

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.

Materials Supplied

Item	Quantity
Plate Fixative Solution	10 mL
Assay Buffer	100 mL
Staining Dye (Avoid light)	10 µl
Inhibitor (U-18666A, 2.5 mM)	10 µl

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 6-well, 12-well, 24-well or 96-well plate
- 100% Ethanol
- 37°C Incubator with 5% CO₂
- Light and fluorescence microscope with UV filter set Ex/Em = 340-380/385-470 nm.

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.

Sample Preparation

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

Cell Culture: Seed 2-3 x 10⁴ cells/well in a 96-well plate in desired media. Grow cells overnight in 37°C incubator containing 5% CO₂. 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.

Assay Procedure

- Bring all reagents and samples to room temperature 30 minutes prior to the assay.
- It is recommended that all standards and samples be run at least in duplicate.
- A standard curve should be run for each assay.

1. 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).

2. 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 photo bleaches very rapidly, we recommend analyzing samples immediately

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:

www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus

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