

**Cholesterol Cell-Based
Detection Assay Kit**

Item No. 10009779

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -20°C kit. For best results, remove components and store as stated below.

Item Number	Item	100 Tests Quantity	Storage
10009899	Cell-Based Assay Fixative	2 vials	Room Temperature
10009866	Cholesterol Detection Wash Buffer	1 vial	Room Temperature
10009867	Cholesterol Detection Filipin III	1 vial	-20°C
10009868	Cholesterol Detection Assay Buffer	1 vial	Room Temperature
10009869	Cell-Based Assay U-18666A	1 vial	-20°C

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. We cannot accept any returns without prior authorization.



WARNING: This product is for laboratory research use only; not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay.

For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

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

INTRODUCTION

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.¹ 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.² 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.^{3,4} 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.⁵ Filipin's ease of use makes it a convenient tool for the histochemical identification of unesterified cholesterol both *in vitro* and *in vivo*.

About This Assay

Cayman's Cholesterol Cell-Based Detection Assay Kit 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.

Reagent 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 (Item No. 10009867) in 200 μ l 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.*

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

Treatment of the Cells

1. Seed a 96-well plate with 3×10^4 cells/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 (Item No. 10009869), 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 μ M.
3. Examine cholesterol localization using the following staining procedure, on page 8.

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 (Item No. 10009899) for 10 minutes.
3. Wash the cells with Cholesterol Detection Wash Buffer (Item No. 10009866), three times, for five minutes each.
4. Dilute the Filipin III Stock Solution (prepared above as described on page 6) 1:100 in Cholesterol Detection Assay Buffer (Item No. 10009868). Add 100 μ l 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.

PERFORMANCE CHARACTERISTICS

Typical Staining Results

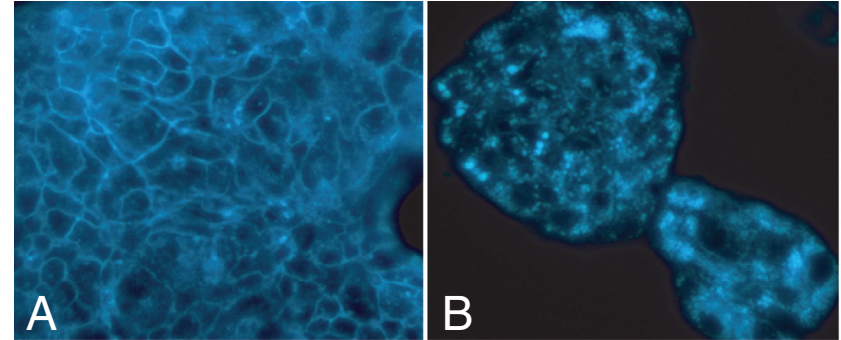


Figure 1: Accumulation of cholesterol inside HepG2 cells in response to 1.25 μ M U-18666A. HepG2 cells were seeded in a 96-well plate at a density of 3×10^4 cells/well and cultured overnight. The next day, cells were treated with DMSO (vehicle) or 1.25 μ M U-18666A for 48 hours. *Panel A:* Cells treated 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 of cholesterol droplets, indicating blockage of intracellular cholesterol transport.

References

1. Mukherjee, S., Zha, X., Tabas, I., *et al.* Cholesterol distribution in living cells: Fluorescence imaging using dehydrogosterol as a fluorescent cholesterol analog. *Biophys. J.* **75**, 1915-1925 (1998).
2. Soccio, R.E. and Breslow, J.L. Intracellular cholesterol transport. *Arterioscler. Thromb. Vasc. Biol.* **24**, 1150-1160 (2004).
3. Miller, R.G. Mini Review. The use and abuse of filipin to localize cholesterol in membranes. *Cell Biology International Reports* **8(7)**, 519-535 (1984).
4. Drabikowski, W., Lagwinska, E., and Sarzala, M.G. Filipin as a fluorescent probe for the location of cholesterol in the membranes of fragmented sarcoplasmic reticulum. *Biochim. Biophys. Acta* **291**, 61-70 (1973).
5. Castanho, M.A.R.B., Coutinho, A., and Prieto, M.J.E. Absorption and fluorescence spectra of polyene antibiotics in the presence of cholesterol. *J. Biol. Chem.* **267**, 204-209 (1992).

Related Products

Adipogenesis Assay Kit - Item No. 10006908

Cholesterol Assay Kit - Item No. 10007640

Glycerol Assay Kit - Item No. 10010755

LDH Cytotoxicity Assay Kit - Item No. 10008882

Phosphatidylcholine Assay Kit - Item No. 10009926

Sphingomyelin Assay Kit - Item No. 10009928

SREBP-2 Transcription Factor Assay Kit - Item No. 10007819

WST-1 Cell Proliferation Assay Kit - Item No. 10008883

Warranty and Limitation of Remedy

Cayman Chemical Company makes no warranty or guarantee of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman warrants only to the original customer that the material **will meet our specifications at the time of delivery**. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have any obligation or liability, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's exclusive remedy and Cayman's sole liability hereunder shall be limited to a **refund** of the purchase price, or at Cayman's option, the **replacement**, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.

NOTES

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