MA-0167

Cholesterol Detection Kit (cell-based)

(100 assays; Light and Fluorescence Microscopy (Ex/Em = 340-380/385-470 nm), Store at -20°C)

Background:

Cholesterol is a vital and versatile structural component of eukaryotic cell membranes, with a fundamental role in maintaining membrane structural integrity and fluidity. Plasma membranes contain most of the cellular cholesterol with little present in the endoplasmic reticulum or mitochondrial membrane. Defects in intracellular cholesterol transport between compartments can alter cellular cholesterol metabolism resulting in pathological conditions such as atherosclerosis and lipid storage diseases. Filipin III selectively binds to and fluorescently labels cholesterol in membranes, emitting a blue fluorescence. AkrivisBio's Cholesterol Detection Kit provides a simple, sensitive method of localizing unesterified cholesterol in cells.

Assay Principle:

Filipin III has a specific binding site for cholesterol which alters the conformation of filipin III activating it's fluorescent properties.

Assay Components:

Fixative Solution	10 ml	WM	MA-0167A
PBS	100 ml	NM	MA-0167B
Filipin III Solution	10 µl	Red	MA-0167C
U-18666A Inhibitor	10 µl	Blue	MA-0167D

User Supplied Reagents & Equipment:

100% Ethanol
37°C CO_2 Incubator
Light and fluorescence microscope with UV filter set Ex/Em = 360-365 /440-480 nm.

Storage and Handling Considerations:

Store unopened kit at -20°C. Open reagents under sterile conditions such as a cell culture hood/biological safety cabinet.

- **Fixative Solution:** Store at room temperature.
- **PBS:** Store at 4°C. Warm to 37°C before use.
- **Staining Dye:** Light sensitive, Use under subdued lighting. Dissolve in 200 µl of pure 100% ethanol (not provided). Aliquot and store at -80°C.
- **Inhibitor (U-18666A):** Store at -20°C. Dilute in PBS as per the assay requirement.

Detection Protocol:

The protocol below is for a 96-well plate. Adjust volumes for larger wells (48 well plate 2X volume; 24 well plate 4X volume).

1. **Cell Culture:** Seed 2-3 x 10^4 cells/well in a 96-well plate. Grow cells overnight in an incubator at 37°C with 5% CO_2. On the following day, treat cells with test compounds in 100 µl media. As a control, use untreated cells. As an inhibitor control, treat cells with U-18666A (concentration depends on cell type, for HepG2 use 1.25 µM. Grow cells for 48-72 hours.
2. **Cell Staining:** Remove culture media from wells. Wash cells with 100 µl PBS. Add 50-100 µl of Fixative Solution and incubate for 10 min. Remove fixative and wash cells with 100 µl PBS 3 times. Dilute Filipin III 1:100 in PBS immediately before using. Add 100 µl/well then incubate in the dark for 30-60 min at 37°C. Carefully remove the filipin III solution using a pipette without disturbing the cells. Gently wash the cells 3 times with 100 µl PBS.

   Note: Cells can be stained directly with filipin III without fixing.
3. **Detection:** Examine cells using light and fluorescence microscope (Ex/Em = 360-365/440-480 nm). Acquire several images per well for analysis.

   Note: Staining Dye photobleaches very rapidly, Analyze samples immediately. Work quickly and efficiently. Avoid exposure to unnecessary light.

FOR RESEARCH USE ONLY! Not to be used for diagnostic or therapeutic purposes.

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