ABSbio™ Cholesterol Uptake Assay Kit

(Cat# K928-200; 200 assays; store kit at -20℃)

Introduction

Cholesterol is one of the major constituents of cellular membranes and is a critical precursor molecule for the synthesis of steroid hormones. Maintaining body cholesterol homeostasis is critical for normal physiological functions and is achieved by a highly regulated balance of de novo synthesis, dietary cholesterol absorption, biliary clearance, and excretion. However, the mechanisms controlling cholesterol absorption are not well understood. Defects in the transport of intracellular cholesterol can alter cellular cholesterol metabolism resulting in pathological conditions. Increased cholesterol uptake has also been linked to highly proliferative cancer cells.

ABSbio's Cholesterol Uptake Assay Kit provides a convenient tool for studying cholesterol absorption modulators, screening cholesterol intracellular trafficking inhibitor and evaluation of effect of drugs on cholesterol uptake. The kit employs a fluorescently-tagged cholesterol, as a probe for the detection of cholesterol uptake by cultured cells. A cholesterol intracellular trafficking inhibitor (U-18666A) is included as a positive control. The fluorescence intensity at Ex/Em=485/535nm, is directly proportional to the amount of fluorescently-tagged cholesterol which is taken up cells. The kit is supplied with sufficient reagents for 200 tests in 96-well plate assay. It could easily be modified for high-throughput assay. The cells also can be measured by fluorescent microscopy and flow cytometry.

Kit Components (200 tests)

Assay Buffer: 30 mL Fluorescently-tagged Cholesterol: 0.4 mL Positive Control: 0.05 mL

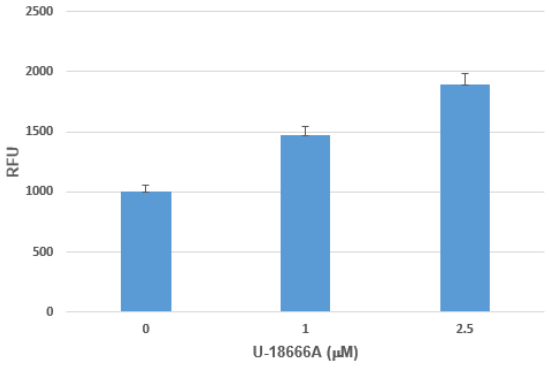
Storage and Handling: Store kit at -20℃. Shelf Life: 6 months after receipt. Warm up Reagents to room temperature before use.

Protocol

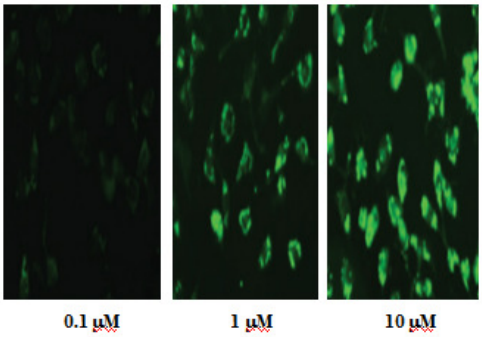
Fluorescent Plate Reader Assay

1. Plate cells at 2-5 x 10^4 cell/well in 100 µL culture medium in a 96-well black, clear bottom culture plate. Grow cells overnight.
2. The next day, wash cell twice with 100 µL PBS, treat the cells with compounds or vehicle control in 100 µL serum free media (or <1% FBS media) containing 20 µg/mL Fluorescently-tagged Cholesterol (F-Ch) (run duplicate or triplicate experiments). Incubate the cells for 48-72 hours. To use the Positive Control, dilute 1:1000 in culture media.
3. Aspirate the culture media.
4. Rinse cells twice with 100 µL PBS.
5. Add 100 µL Assay Buffer to each well.
6. Immediately read fluorescence at Ex/Em=485/535 nm.
7. Compare fluorescence intensity of treatment relative to controls.

Cholesterol uptake results



Fluorescently-tagged Cholesterol uptake by Caco-2 cells treated with various concentrations of Positive Control



Representative images of THP-1 cells treated with various concentrations of fluorescent cholesterol ranging (0.1, 1 & 10 µM).

Fluorescent Microscopy Assay

1. Plate cells at 2-5 x 10^4 cell/well in 100 µL culture medium in a 96-well black, clear bottom culture plate. Grow cells overnight.
2. The next day, wash cell twice with 100 µL PBS, treat the cells with compounds or vehicle control in 100 µL serum free media (or <1% FBS media) containing 20 µg/mL Fluorescently-tagged Cholesterol (F-Ch) (run

duplicate or triplicate experiments). Incubate the cells for 48-72 hours. To use the Positive Control, dilute 1:1000 in culture media.

3. Aspirate the culture media.
4. Rinse cells twice with 100 µL PBS.
5. Add 100 µL PBS to each well. Be careful to not disturb the cells.
6. Immediately analysis by fluorescent microscopy at Ex/Em=485/535 nm.
7. Compare fluorescence intensity of treatment relative to controls.

Flow Cytometry Assay

1. Plate cells at 5 x 10^5 cells/well in 6- or 12-well culture plate. Grow cells overnight.
2. The next day, wash cell twice with PBS, treat the cells with compounds or vehicle control in serum free media (or <1% FBS media) containing 20 µg/mL Fluorescently-tagged Cholesterol (F-Ch) (run duplicate or triplicate experiments). Incubate the cells for 48-72 hours. To use the Positive Control, dilute 1:1000 in culture media.
3. At the end of the treatment, harvest cells from each well into a tube for the flow cytometer.
4. Resuspend the cells with 500 µL PBS. Mix well to ensure separation of individual cells.
5. Immediately analyze the cells with a flow cytometer at Ex/Em=485/535 nm.

References

Zhang, J. et al. 2011, ASSAY and Drug Development Technologies, 9:136-146
Song, W. et al. 2015, Molecular Medicine Report, 12: 5989-5996
Feng, D., et al 2010, Lipids Health Dis 9: 40.

Related Products:

Glucose Uptake Assay Kit (#K958-100)