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Product Information

Cholesterol Uptake Assay Kit

Catalog Number **MAK325**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Cholesterol is a sterol and lipid present in cell membranes, and is transported in the bloodstream of all animals. It is used to form cell membranes and hormones, and plays important roles in cell signaling processes. Cellular regulation of cholesterol levels is a complex system in which irregularities have been tied to obesity and heart disease. Increased cholesterol uptake has also been linked to highly proliferative cancer cells. Through monitoring cellular cholesterol uptake, one can explore these growing health problems and screen for possible drug treatments.

The Cholesterol Uptake Assay Kit is based on cellular uptake of a fluorescently tagged cholesterol probe. The fluorescence intensity measured at λ_{ex} = 485 nm/ λ_{em} = 535 nm is proportional to the amount of cholesterol uptaken by the cells.

This kit is suitable for determination of cholesterol uptake by adherent cells, screening of cholesterol uptake inhibitors, and evaluation of effect of drugs on cholesterol uptake.

Components

The kit is sufficient for 100 fluorometric assays in 96 well plates.

Assay Reagent Catalog Number MAK325A	12 mL
Fluorescent Tracer Catalog Number MAK325B	250 µL
Positive Control (2.5 mM) Catalog Number MAK325C	20 µL

Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Black flat bottom 96-well plates
- Culture medium
- Phosphate buffered saline (PBS), pH 7.4, prepared from Catalog Number P3813 (powder) or P4417 (tablets)
- Centrifuge tubes
- Fluorescence plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Reagent Preparation

Equilibrate all reagents to room temperature. Briefly centrifuge tubes before use.

Dilute Fluorescent Tracer 50-fold in serum free medium or low percentage (<1%) FBS medium. Add any treatments or compounds being tested to the culture medium.

To use the Positive Control, dilute 1,000-fold in culture medium with Fluorescent Tracer for a final concentration of 2.5 μM. Various concentrations of Positive Control may need to be tested to determine the most effective concentration for the cell line being used.

Storage/Stability
The kit is shipped at room temperature. Store components at –20 °C upon receiving.

Procedure
Cell Preparation
Test Samples: Plate cells at desired density in 100 μL of culture medium with Fluorescent Tracer and supplemented with any test compound (e.g., inhibitor) being evaluated in a black flat bottom 96 well plate.

Controls: Plate additional set of cells at desired density in 100 μL of culture medium with Fluorescent Tracer in a black flat bottom 96 well plate. Omit any test compounds from the Controls.

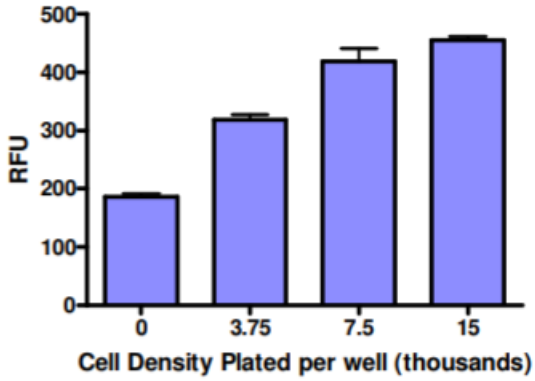
If using Positive Control, plate a second set of control cells in 100 μL of culture medium with Fluorescent Tracer and 2.5 μM Positive Control (or other concentration of Positive Control, see Preparation Instructions) in a black flat bottom 96 well plate.

Allow cells to propagate for 24–72 hours or to desired confluence.
Note: It is strongly recommended to run all Test Samples and Controls in duplicate at a minimum; running Test Samples and Controls in triplicate or more is desirable.

Assay Reaction
1. Carefully aspirate culture medium from all wells.
2. Rinse all wells twice with 100 μL of 1× PBS. Be sure to remove all PBS when finished.
3. Add 100 μL of Assay Reagent to all wells.
4. Read fluorescence at λ_{ex} = 485 nm/ λ_{em} = 535 nm. No incubation period is required.

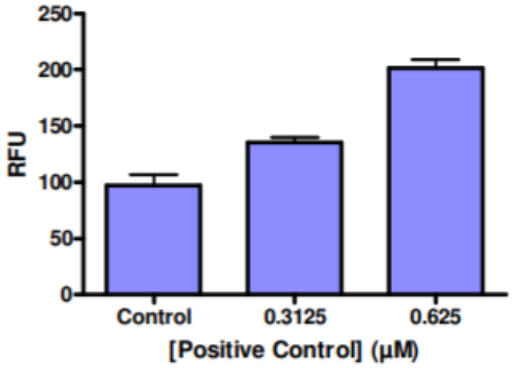
Results
Compare fluorescence intensity of treatment relative to controls. Wells with greater fluorescence indicate an increase in cholesterol uptake. Wells with lower fluorescence indicate a decrease in cholesterol uptake.

Figure 1.
Cell Density and Cholesterol Uptake



PANC1 cells seeded at varying cell densities in 1% FBS DMEM with Fluorescent Tracer. Propagated for 48 hours before assay

Figure 2.
Positive Control



MDA-MB-231 cells treated with varying concentrations of Positive Control in Serum Free medium with Fluorescent Tracer. Propagated 72 hours prior to assay.

References
1. Beloribi-Djefaflia, S. et al., Lipid metabolic reprogramming in cancer cells. Oncogenesis, **5**, e189 (2016).
2. Martin, B., and van Golen, K. A., Comparison of Cholesterol Uptake and Storage in Inflammatory and Noninflammatory Breast Cancer Cells. Int. J. Breast Cancer, Article ID 412581 (2012).
3. Feng, D. et al., Curcumin inhibits cholesterol uptake in Caco-2 cells by down-regulation of NPC1L1. Lipids Health Dis., 9, 40 (2010).

VNC,HM,MAM 11/18-1