Cholesterol Uptake Assay Kit (Fluorometric)

Cholesterol Uptake Assay Kit (Fluorometric) is a detection kit for the quantification of Cholesterol Uptake in adherent cells.

Catalog number: ARG82144

Package: 100 tests

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INTRODUCTION

Cholesterol (from the Ancient Greek chole- (bile) and stereos (solid), followed by the chemical suffix *-ol* for an alcohol) is an organic molecule. It is a sterol (or modified steroid), a type of lipid. Cholesterol is biosynthesized by all animal cells and is an essential structural component of animal cell membranes. It is a yellowish crystalline solid.

Cholesterol also serves as a precursor for the biosynthesis of steroid hormones, bile acid and vitamin D. Cholesterol is the principal sterol synthesized by all animals. In vertebrates, hepatic cells typically produce the greatest amounts. It is absent among prokaryotes (bacteria and archaea), although there are some exceptions, such as Mycoplasma, which require cholesterol for growth. [Provide by Wikipedia: Cholesterol]

PRINCIPLE OF THE ASSAY

This Cholesterol Uptake Assay Kit (Fluorometric) is a simple fluorometric assay that measures the amount of Cholesterol present in adherent cell sample. This kit is based on cellular uptake of a fluorescently tagged cholesterol probe. The fluorescence intensity measured at λex/em = 485/535 nm is proportional to the amount of cholesterol uptaken by the cells.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped at room temperature. Store all components at -20°C. Shelf life of 6 months after receipt.

Component	Quantity	Storage information
Assay Reagent	12 mL	-20°C
Fluorescent Tracer	250 µL	-20°C
Positive Control (2.5 mM)	120 µL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Fluorescence microplate reader capable of reading excitation at 485 nm and emission at 535 nm.
- Centrifuge
- Culture medium and 1X PBS
- Black flat-bottom 96 well microplate
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

Adherent cells: assay directly.

REAGENT PREPARATION

- Tracer Medium: Prepare Tracer Medium by diluting Fluorescent Tracer 1:50 in serum free media or low percentage FBS media (<1%). You will use Tracer Medium to prepare media for treatment, control, and positive controls.

ASSAY PROCEDURE

Prior to the assay, equilibrate all components to room temperature. Briefly centrifuge tubes before opening.

Note: we recommend running all experimental variables in at least duplicate if not triplicate or greater.

1. Use a fluorescent, flat-bottom, black 96-well microplate for assay.
2. For **control** well, seed cells at desired density in **100 µL** of **Tracer Medium**.
3. For the **positive control**, in a separate tube dilute the **2.5 mM Positive Control 1:1000** in **Tracer Medium** for a final concentration of **2.5 µM**. Seed cells at desired density in **100 µL** of the **positive control spiked tracer medium**.
4. For each **treatment**, in a separate tube spike the treatment or compound at the desired concentration into Tracer Medium. Seed cells at desired density in **100 µL** of **treatment spiked Tracer Medium**.
5. Allow cells to propagate for **24 to 72 hours** or to desired confluence.
6. Carefully aspirate culture medium from all wells.
7. Rinse all wells twice with **100 µL** of **1XPBS**. Be sure to remove all PBS when finished.
8. Add **100 µL** of **Assay Reagent** to all wells.
9. Read the plate immediately with a fluorescence microplate reader using **excitation 485 nm filter** and **emission 535 nm filter.**

CALCULATION OF RESULTS

1. 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.

EXAMPLE OF RESULT

The following figures demonstrate typical results with the Cholesterol Uptake Assay Kit (Fluorometric). One should use the data below for reference only. This data should not be used to interpret actual results.