

Cholesterol Uptake Assay Kit (Colorimetric/Fluorometric)

LS-K162-100 (100 Tests) • Store at -20°C



Introduction

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. LSBio's cholesterol uptake assay kit is based on cellular uptake of a fluorescently tagged cholesterol probe. The fluorescence intensity measured at $\lambda_{ex}/\lambda_{em} = 485/535$ nm is proportional to the amount of cholesterol taken up by the cells.

Key Features

- Convenient. Treat cells directly in 96-well fluorescent plate.
- Safe. Non-radioactive assay.
- High-throughput. Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

Applications

- **Direct Assays:** Cholesterol uptake by adherent cells, screening of cholesterol uptake inhibitors, and evaluation of effect of drugs on cholesterol uptake.

Components

| Component | K162-100 |
|--------------------|-------------|
| | 100 Tests |
| Assay Reagent | 12 mL |
| Fluorescent Tracer | 250 μ L |
| Positive Control | 20 μ L |

Materials Not Supplied

Pipetting devices, culture medium, PBS, black flat-bottom 96-well plates, and fluorescent plate reader capable of reading at $\lambda_{ex}/\lambda_{em} = 485/535$ nm.

Storage

The kit is shipped at room temperature. Store all components at -20°C upon receiving.

FOR RESEARCH USE ONLY! Not for use in humans.

LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121
www.LSBio.com • (206) 464-1554 • TechnicalSupport@LSBio.com

Cholesterol Uptake Assay Kit (Colorimetric/Fluorometric)

LS-K162-100 (100 Tests) • Store at -20°C



Assay Procedure

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

To use the Positive Control, dilute 1:1000 in culture medium for a final concentration of 2.5 μM . You may need to test various concentrations of Positive Control to determine the most effective for the cell line being used.

Plate cells at desired density in 100 μL culture medium with Fluorescent Tracer and any treatments being tested in a black flat-bottom 96-well plate (we recommend running all experimental variables in at least duplicate if not triplicate or greater). Allow cells to propagate for 24 to 72 hours or to desired confluence.

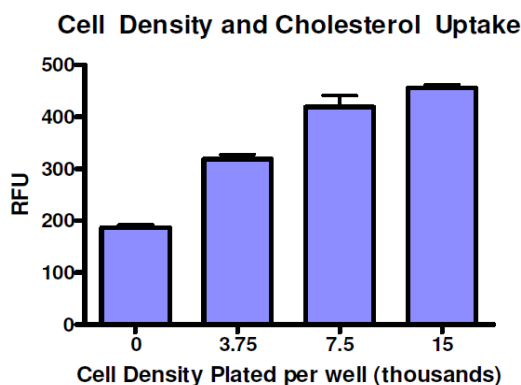
Assay Procedure using 96-well plate

1. Carefully aspirate culture medium from all wells.
2. Rinse all wells twice with 100 μL 1_x PBS. Be sure to remove all PBS when finished.
3. Add 100 μL Assay Reagent to all wells.
4. Read fluorescence at $\lambda_{\text{ex/em}} = 485/535 \text{ nm}$.

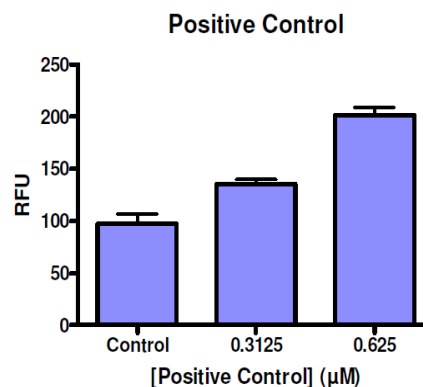
Data Analysis

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.

Sample Data



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



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

Version: V.08.09.2018

FOR RESEARCH USE ONLY! Not for use in humans.

LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121

www.LSBio.com • (206) 464-1554 • TechnicalSupport@LSBio.com