

QuantiFluo™ Cholesterol Uptake Assay Kit (DCUT-100)

Quantitative Fluorimetric Determination of Cholesterol Uptake

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.

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

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Reagent:	12 mL
Fluorescent Tracer:	250 μ L
Positive Control (2.5 mM):	20 μ L

Storage conditions. The kit is shipped at room temperature. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Precautions: 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.

Reagent Preparation: Equilibrate all components to room temperature prior assay. Briefly centrifuge tubes before opening.

PROCEDURES

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

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

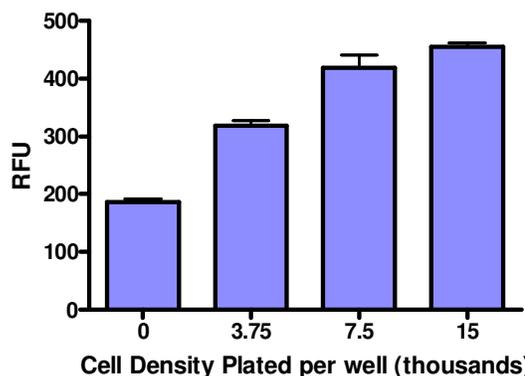
DATA ANALYSIS

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.

MATERIALS REQUIRED, BUT NOT PROVIDED

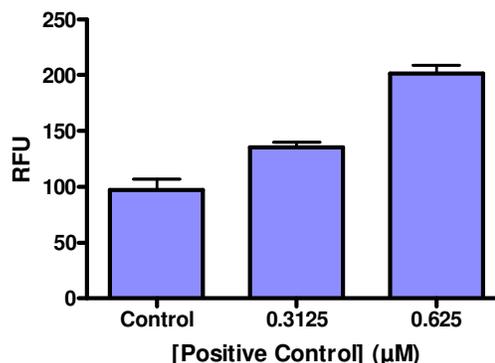
Pipetting devices, culture medium, PBS, black flat-bottom 96-well plates, and fluorescent plate reader capable of reading at $\lambda_{ex/em} = 485/535$ nm.

Cell Density and Cholesterol Uptake



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.

Positive Control



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.

LITERATURE

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