# 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 µL |
| **Positive Control (2.5 mM):** | 20 µL |

**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

**Tracer Medium Preparation:** 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 using 96-well plate**

1. Use a fluorescent, flat-bottom, black 96-well plate for assay.

For control wells, seed cells at desired density in 100 µL tracer medium.

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. *Note: you may need to test various concentrations of Positive Control to determine the most effective for the cell line being used.*

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 treatment spiked Tracer Medium.

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

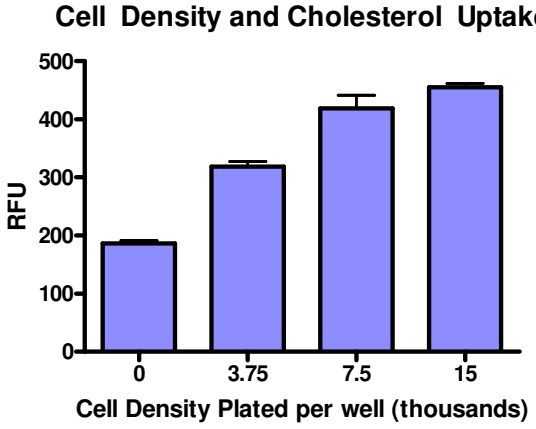
2. Allow cells to propagate for 24 to 72 hours or to desired confluence.
3. Carefully aspirate culture medium from all wells.
4. Rinse all wells twice with 100 µL 1× PBS. Be sure to remove all PBS when finished.
5. Add 100 µL Assay Reagent to all wells.
6. Read fluorescence at $\lambda_{ex/em}$ = 485/535 nm immediately.

## DATA ANALYSIS

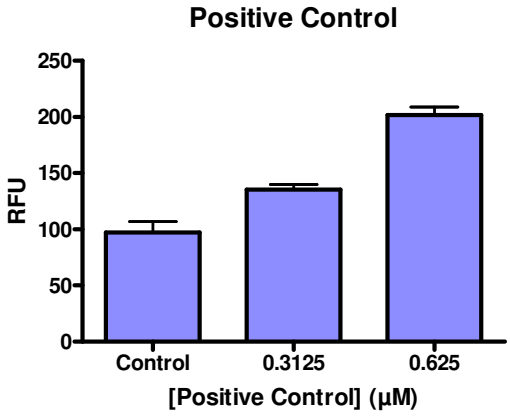
Compare fluorescence intensity of treatment relative to controls. Wells with greater fluorescence indicate an increase in cholesterol uptake. Wells with lower fluorescence indicate a decrease in cholesterol uptake.

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




**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

1. Beloribi-Djefaflia, S., et al (2016). Lipid metabolic reprogramming in cancer cells. Oncogenesis 5, e189.
2. 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.
3. Feng, D., et al (2010). Curcumin inhibits cholesterol uptake in Caco-2 cells by down-regulation of NPC1L1. Lipids Health Dis 9: 40.