

Technical Manual

FluoroDazzle Cholesterol Uptake Assay Kit

Catalogue Code: BA0021

Pack Size: 100 assays

Research Use Only

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 Assay Genie FluoroDazzle 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.

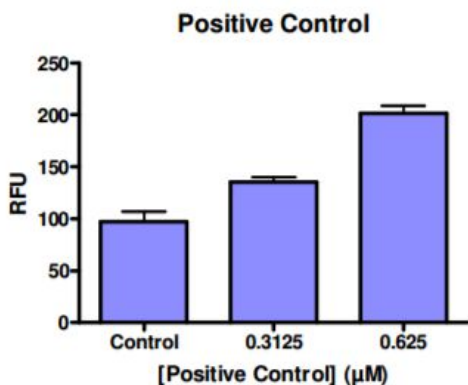
PROCEDURES

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 μ M. 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 μ L 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

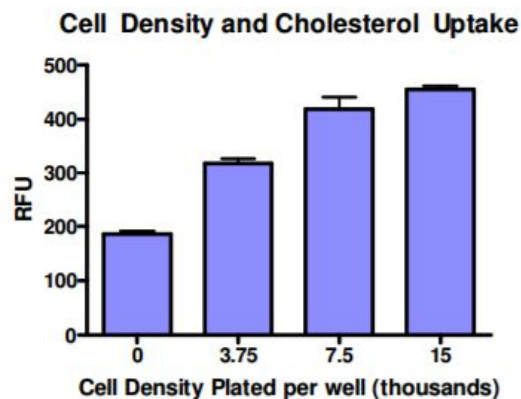
1. Carefully aspirate culture medium from all wells.
2. Rinse all wells twice with 100 μ L 1X PBS. Be sure to remove all PBS when finished.
3. Add 100 μ L Assay Reagent to all wells.
4. Read fluorescence at $\lambda_{ex/em} = 485/535$ nm.

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.



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.



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.

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

Contact Details

Dublin, Ireland

Email: info@assaygenie.com

Web: www.assaygenie.com

Technical Support: Techsupport@assaygenie.com