Cholesterol Uptake Cell-Based Assay Kit

Item No. 600440

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -20°C kit. For best results, remove components and store as stated below.

Item Number	Item	Quantity/Size	Storage
600441	Cell-Based Assay NBD Cholesterol	1 vial/500 µl	-20°C
10009322	Cell-Based Assay Buffer Tablet	1 vial/1 tablet	RT
10009869	Cell-Based Assay U-18666A	1 vial/100 µl	-20°C

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3640

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. Tissue culture plates for culturing cells (black, clear bottom if using a plate reader)
2. Cells that will take up cholesterol, and the appropriate serum-free medium for the assay
3. A flow cytometer, microscope, or plate reader capable of detecting fluorescence at excitation and emission wavelengths of 485 nm and 535 nm, respectively

INTRODUCTION

About This Assay

Cayman's Cholesterol Uptake Cell-based Assay Kit provides a convenient tool for studying cellular cholesterol trafficking. Maintenance of the balance between cholesterol synthesis, efflux, and uptake is tightly regulated and dysregulation is associated with disorders such as obesity and atherosclerosis.[1,2] The kit employs NBD Cholesterol, a fluorescently tagged cholesterol, as a probe for the detection of cholesterol taken up by cultured cells. U-18666A, which increases cholesterol uptake by inhibiting trafficking of synthesized cholesterol, is included as a positive control. This kit provides enough NBD Cholesterol to test 250 samples in a 96-well format.

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Cell-Based Assay Buffer - (Item No. 10009322)**

   Dissolve the Cell-Based Assay Buffer Tablet (Item No. 10009322) in 100 ml of distilled water. This buffer should be stable for approximately one year at room temperature.

2. **Cell-Based Assay NBD Cholesterol - (Item No. 600441)**

   This fluorescently tagged cholesterol derivative is supplied as a solution in ethanol at 1 mg/ml. Dilute this solution 1:50 in the serum-free culture medium used for your experiments. The final concentration of NBD Cholesterol in the culture medium is 20 µg/ml.

   NOTE: Protect from light.

3. **Cell-based Assay U-18666A - (Item No. 10009869)**

   This cholesterol transport inhibitor is provided at a concentration of 2.5 mM in DMSO.

ASSAY PROTOCOL

Procedure

1. Culture cells as your experiment requires: for plate reader detection, a black clear-bottom 96-well plate is recommended; for microscopy or flow cytometry, any size wells can be used for culture. Flow cytometric readout is ideal for suspension cells, while plate reader or microscopy are better suited for adherent cell lines. While optimal cell numbers must be determined for each application, cells should not be more than 80% confluent by the end of the assay.
2. Treat the cells with experimental compounds or vehicle control in 100 µl serum-free culture medium containing 20 µg/ml NBD Cholesterol (see page 6). Incubate the cells for 24-72 hours. To use the included U-18666A as a positive control, dilute 1:1,000 - 1:4,000 in serum-free culture medium.
3. *For flow cytometry*: Collect cells into FACS tubes or v-bottom plates. Centrifuge at 250 x g for 5 minutes, and remove supernatant. Add 100-500 µl Assay Buffer and analyze with the flow cytometer immediately, typically using an FL1 (FITC) channel.
4. *For microscopy or plate reader*: remove medium and replace with an appropriate volume of Assay Buffer. Analyze immediately, with filter sets designed for FITC/GFP.

PERFORMANCE CHARACTERISTICS

Flow Cytometry

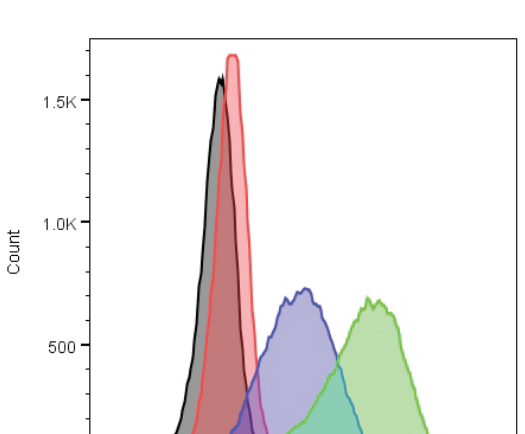

Figure 1. U-18666A increases cholesterol uptake in Jurkat cells as measured by flow cytometry. Jurkat cells were seeded at a density of 5 x 10^5 cells/ml and incubated overnight in serum-free RPMI with U-18666A or vehicle and 20 µg/ml NBD Cholesterol in a cell culture incubator at 37°C. The next day, cells were transferred to a v-bottom plate for washing and reading on a flow cytometer. Cholesterol uptake was evaluated in the live cell gate using FlowJo analysis software. U-18666A at both 2.5 µM (green) and 1.25 µM (blue) showed a significant ($p<0.05$, t-test) shift in mean fluorescence as compared to the vehicle control (black) and the untreated (red) cells.

Fluorescence Microscopy

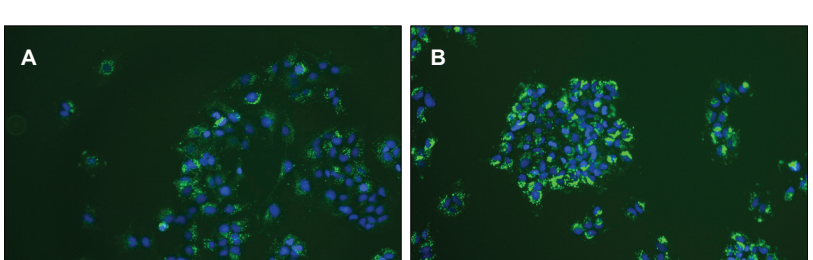

Figure 2. Blocking intracellular cholesterol transport with U-18666A increases NBD cholesterol uptake. Huh-7 hepatocytes seeded at 3 x 10^3 cells/well were treated overnight with (*Panel B*) or without (*Panel A*) 1.25 µM U-18666A in the presence of 20 µg/ml NBD cholesterol in serum-free media. Hoechst (Item No. 600332) was added to a final concentration of 4 µM for the final 30 minutes, after which media was exchanged for Assay Buffer. Cells were imaged using a Cytation™ 5 Cell Imaging Multi-Mode Reader (BioTek Instruments, Inc.), using a GFP LED/filter set for NBD cholesterol (green) and a DAPI LED/filter set for Hoechst (blue).

Plate Reader

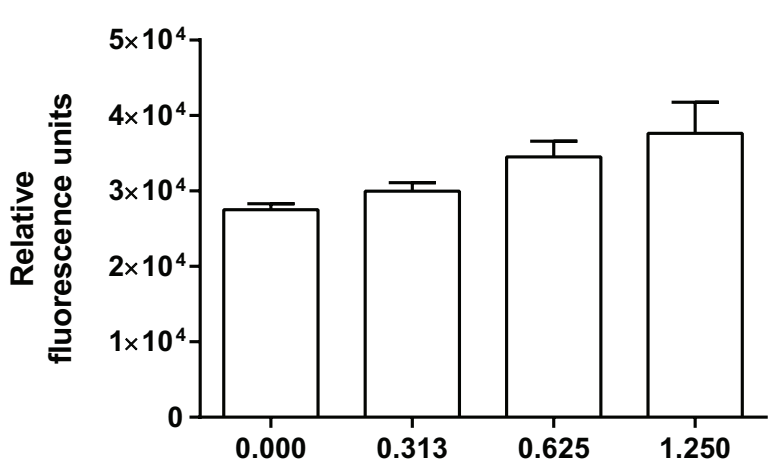

Figure 3. U-18666A causes a dose-dependent increase in NBD Cholesterol uptake in Caco-2 cells, as measured on a fluorescent plate reader. Caco-2 cells were seeded at a density of 10,000 cells/well and incubated overnight at 37°C. The next day, cells were treated with vehicle or the indicated concentrations of U-18666A in serum-free culture medium with 20 µg/ml NBD Cholesterol for three days. At the end of the experiment, the degree of NBD Cholesterol uptake was analyzed using a plate reader.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
No cholesterol uptake in all treatments, including positive control	Cells are not healthy	Use only healthy cells
No significant difference in fluorescent staining intensity among treatments	Culture medium contains high level of serum	Use culture medium which contains no serum

References

1. Soccio, R.E. and Breslow, J.L. *Arterioscler. Thromb. Vasc. Biol.* **24**, 1150-1160 (2004).
2. Santosa, S., Varady, K.A., AbuMweis, S., *et al. Life Sci.* **80(6)**, 505-514 (2007).

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.