LDL Uptake Flow Cytometry Assay Kit

Item No. 601470

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Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a 4°C kit. After opening the kit, store individual components as stated below.

Item Number	Item	Quantity/Size	Storage
601471	LDL-DyLight™ 488	1 vial	4°C
601472	Lovastatin Control	1 vial/0.1 mg	4°C
10009322	Cell-Based Assay Tablet	1 tablet	RT
400201	7-AAD Viability Dye (1,000X)	1 vial/50 µl	4°C

NOTE: DyLight™ 488 is a product of Thermo Fisher Scientific Inc.
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: 734-971-3641

E-Mail: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

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. Cells that take up LDL (*e.g.* HepG2 or Huh-7 cell lines), appropriate medium and cell dissociation reagents.
2. Recommended: Medium with low serum or lipoprotein-depleted serum
3. DMSO
4 Flow cytometer equipped with a 488 nm laser line and filters capable of detecting 525 nm and 650-700 nm
5. FACS tubes or v-bottom staining plates

INTRODUCTION

Background

Cholesterol is an essential cellular component and maintenance of cholesterol homeostasis is critical for normal physiological functions. However, elevated levels of plasma cholesterol are associated with various pathological conditions, most notably atherosclerosis, in which elevated low density lipoprotein (LDL) cholesterol levels are associated with foam cell formation and plaque formation in arteries.[1] The resulting heart attacks, strokes and other related illnesses accounted for 32% of deaths and 17% of healthcare spending in the US in 2009.[2] Thus, circulating LDL is a high-value target for pharmaceutical intervention in cardiovascular disease.

LDL is the major carrier of cholesterol in the blood, accounting for more than 60% of total plasma cholesterol. Regulation of plasma cholesterol levels and cellular cholesterol metabolism depends on a balance of LDL receptor (LDLR)-mediated uptake and cellular cholesterol production.[3] LDL is taken up by hepatic and extrahepatic tissues through receptor-mediated endocytosis triggered by a direct interaction between the LDL protein component apoB and LDLR. The balance of cellular cholesterol source can be shifted towards uptake and away from production by pharmacologic intervention.[4] Statins effectively inhibit HMG-CoA reductase activity, which reduces endogenous cholesterol synthesis, along with other pleiotropic effects. This encourages upregulation of surface LDLR and uptake of extracellular cholesterol in the form of LDL, resulting in the depletion of circulating LDL.[5] Statins have proven remarkably effective at diminishing risk of heart attack and other outcomes associated with high LDL, but the sheer numbers of people with cardiovascular disease ensure that cholesterol-lowering drugs will continue to be an important target for some time to come.

About This Assay

Cayman's LDL Uptake Flow Cytometry Assay Kit employs human LDL conjugated to DyLight™ 488 as a convenient tool for studying the uptake of LDL in cultured cells. Flow cytometry provides the advantage of assessing the uptake of LDL at the single-cell level. In addition, multiplexing with other markers, such as LDLR expression, is possible to gain more information from a single experiment. Lovastatin is included in this kit as a control modulator of LDL uptake by hepatocytes. The reagents provided in this kit are sufficient to test 48 samples by flow cytometry.

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Assay Buffer**

   Dissolve tablet in 100 ml distilled water. Store this assay buffer at room temperature for up to one year.

2. **LDL-DyLight™ 488 Working Solution**

   Mix tube gently. *Do not vortex.* Dilute LDL-DyLight™ 488 (Item No. 601471) 1:10 in your culture medium. If particulates are observed, filter diluted LDL-DyLight™ 488 through a 0.45 um syringe-top filter. Dilute immediately before use.

3. **Lovastatin Control**

   Resuspend Lovastatin control (Item No. 601472) in 250 µl DMSO. This stock is a 1 mM solution of lovastatin, which should be stored after use at -20°C for up to 6 months.

4. **7-AAD Staining Solution**

   Dilute the 7-AAD Viability Dye (1,000X) (Item No. 400201) by adding 10 µl into 10 ml of assay buffer. This solution should be used immediately.

ASSAY PROTOCOL

Flow cytometry

This protocol is designed for use in a 24-well plate with 500 µl per well. For different vessel sizes, adjust volumes accordingly.

1. Culture cells and treat as required by your experimental design in a CO_2 incubator at 37°C, running each sample in duplicate or triplicate. Cells should be <80% confluent at the time of staining. Use of low- or no-serum medium is recommended, or medium with LDL-depleted FBS.
2. To use the supplied lovastatin as a control modulator of LDL uptake, dilute to a final concentration of 1 µM in culture medium. Treat cells for 24 hours total with lovastatin to increase LDL uptake.
3. Four hours before the end of the treatment, add 25 µl diluted LDL-Dylight™ 488 (prepared on page 7) to each well, for a final dilution of 1:200. Incubate at 37°C for four hours in the dark.
4. At the end of the incubation, remove cells from culture dish (careful enzymatic removal is compatible with this protocol) to FACS tubes or a v-bottom staining plate. It is recommended to set aside a small number of cells in a separate tube for a compensation control. These cells will not be stained with 7-AAD.
5. Centrifuge cells at 250 x g for five minutes and remove supernatant.
6. Resuspend cells in 200 µl Assay Buffer.
7. Centrifuge cells at 250 x g for five minutes and remove supernatant.
8. Resuspend cells in 100-200 µl 7-AAD Staining Solution prepared on page 7, triturating gently to obtain a single cell suspension. The compensation control cells (step 4) should be resuspended in Assay Buffer.
9. Collect data on the flow cytometer, detecting LDL-DyLight™ 488 in the FITC channel and 7-AAD in the PE or PI/PerCP channel. Some cell types which are ideal for LDL uptake (e.g. HepG2 cells) can be difficult to make into a single-cell suspension, so exclusion of doublets may be necessary for generating robust data.

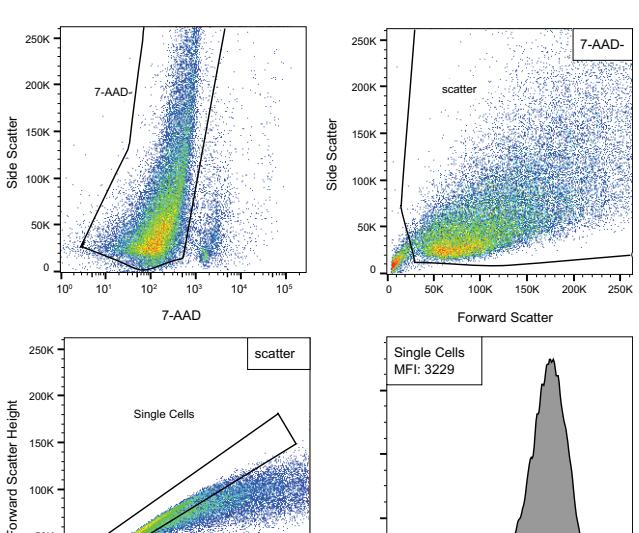

Figure 1: Example flow cytometric analysis. HepG2 cells were plated at 2 x 10^5 cells/well in a 24 well plate approximately 48 hours before addition of LDL-DyLight™ 488. After a four hour incubation with the LDL probe, cells were trypsinized, washed and stained with 7-AAD prior to flow cytometry. In this example analysis, after digital compensation, 7-AAD negative live cells are gated first, followed by scatter. Single cells are gated using an area versus height dot plot, and the geometric mean fluorescence intensity (MFI) of the resulting cells in the LDL-DyLight™ 488 channel is determined.

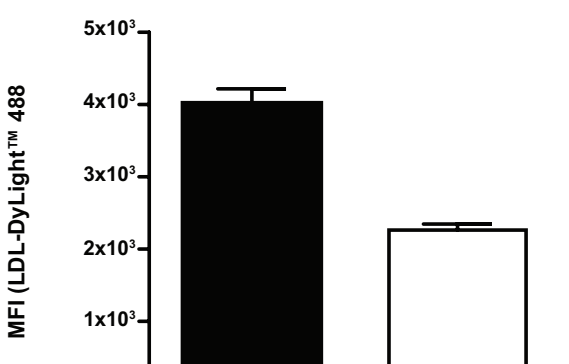

Figure 2: Lovastatin increases uptake of LDL. HepG2 cells were plated at 2 x 10^5 cells/well in a 24 well plate and allowed to adhere overnight, before being treated with 1 µM Lovastatin or left untreated for 24 hours in MEM + 2% FBS. The final four hours of treatment included the probe LDL-DyLight™ 488. Cells were processed as described in the kit booklet and the flow cytometric data were analyzed as described in Figure 1. Average geometric mean fluorescence intensities (MFI) for each group are plotted.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Replicates have varying values	A. LDL aggregates B. LDL sticks to side of cell culture vessel	A. Filter diluted LDL solution through 0.45 µM filter before adding to cells B. Add LDL solution to media directly, not to vessel wall
High 7-AAD staining	A. Treatment kills cells B. Cells not healthy before experiment began C. Cells are compromised during processing	A. Titrate treatment B. Use only healthy cells C. Trypsinize for minimal amount of time, process cells gently.

References

1. Soccio, R.E. and Breslow, J.L. *Arteriosclerosis, Thrombosis, and Vascular Biology* **24**, 1150-1160 (2004).
2. National Heart, Lung and Blood Institute. U.S. Dept. Health Human Services 33-52 (2012).
3. Goldstein, J.L., Brown, M.S., Anderson, R.G.W., *et al. Ann. Rev. Cell Biol.* **1**, 1-39 (1985).
4. Rudling, M.J., Reihnér, E., Einarsson, K., *et al. Proc. Natl. Acad. Sci. USA* **87**, 3469-3473 (1990).
5. Stancu, C. and Sima, A. *J. Cell. Mol. Med.* **5(4)**, 378-387 (2001).

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.