LDL Uptake Cell-Based Assay Kit

Item No. 10011125

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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	100 Tests Quantity	Storage
10009899	Cell-Based Assay Fixative	1 vial	RT
10012422	Rabbit Anti-LDL Receptor Primary Antibody	1 vial	-20°C
10009906	Cell-Based Assay Blocking Solution	1 vial	4°C
10011229	LDL-DyLight™ 550	1 vial	4°C
10011231	DyLight™ 488-Conjugated Goat Anti-Rabbit IgG Secondary Antibody	1 vial	-20°C

NOTE: DyLight™ 550 and DyLight™ 488 are products 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 such as HepG2 (can be obtained from ATCC) and appropriate medium.
2. A 0.45 µm syringe-top filter.
3. TBS, pH 7.4.
4 Triton-X 100.
5. A fluorescent microscope equipped with filter sets capable of detecting fluorescein (excitation/emission = 485/535 nm) and rhodamine (excitation/emission = 540/570 nm).

INTRODUCTION

Background

Cholesterol is an essential cellular component and maintenance of cholesterol homeostasis is critical for normal physiological functions. Elevated levels of plasma cholesterol are associated with various pathological conditions, most notably coronary heart disease where high cholesterol levels lead to foam cell formation and plaque buildup in arteries, potentially resulting in a heart attack or stroke.[1] Regulation of cellular cholesterol metabolism and plasma cholesterol levels depends on low-density lipoprotein (LDL) receptor-mediated LDL uptake into specific cells.[2]

LDL is the major carrier of cholesterol in the blood, accounting for more than 60% of total plasma cholesterol. LDL is taken up by hepatic and extrahepatic tissues through receptor-mediated endocytosis triggered by apoB-100-LDL receptor interaction. The internalized LDL particle is transported to lysosomes where it is degraded to free cholesterol and amino acids. In humans, the liver is the most important organ for LDL catabolism and LDL receptor activity. In the liver, LDL can be regulated by pharmacologic intervention.[3]

About This Assay

Cayman's LDL Uptake Cell-Based Assay Kit provides a convenient tool for studying LDL uptake and regulation at the cellular level. The kit employs human LDL conjugated to DyLight™ 550 (product of Thermo Fisher Scientific Inc.) as a fluorescent probe for detection of LDL uptake into cultured cells. An LDL receptor-specific polyclonal antibody and a DyLight™ 488-conjugated secondary antibody are included for identifying the distribution of LDL receptors.

PRE-ASSAY PREPARATION

NOTE: Reagents #2-4 should be prepared just prior to immunofluorescent staining of LDL receptors.

Reagent Preparation

1. **LDL-DyLight™ 550 Working Solution**

   Dilute the LDL-DyLight™ 550 (Item No. 10011229) 1:100 in appropriate serum-free culture medium. Remove particulates with a 0.45 µm filter before adding to the cells.

2. **TBS-Triton Buffer**

   Prepare TBS-Triton Buffer by adding Triton X-100 to TBS to a final concentration of 0.1%.

3. **Rabbit Anti-LDL Receptor Primary Antibody**

   Dilute the Rabbit Anti-LDL Receptor Primary Antibody (Item No. 10012422) 1:100 in TBS-Triton Buffer.

4. **DyLight™ 488-Conjugated Goat Anti-Rabbit IgG Secondary Antibody**

   Dilute the DyLight™ 488-Conjugated Goat Anti-Rabbit IgG Secondary Antibody (Item No. 10011231) 1:100 in TBS-Triton Buffer.

ASSAY PROTOCOL

Treatment of Cells and Uptake of LDL-DyLight™ 550

The following protocol is designed for a 96-well plate. For other sizes of plates the volume of medium/solution to apply to each well should be adjusted accordingly. We recommend performing each treatment in duplicate or triplicate.

1. Seed a 96-well plate with 3 x 10^4 cells/well and grow cells overnight. For HepG2 cells, grow cells for two days before treatment.
2. The next day or third day, treat cells with experimental compounds or vehicle for 24 hours, or for the period of time used in your typical experimental protocol.
3. At the end of the treatment period, replace the culture medium with 75-100 µl/well LDL-DyLight™ 550 working solution prepared above in serum-free medium. Incubate the cells at 37°C for an additional 3-24 hours, or for the period of time used in your typical experimental protocol.
4. At the end of the LDL uptake incubation, aspirate the culture medium and replace with fresh culture medium or PBS. Examine the degree of LDL uptake under a microscope with filters capable of measuring excitation and emission wavelengths 540 and 570 nm, respectively. The cells are now ready for the immunofluorescent staining of LDL receptors using the procedure described on page 8.

Immunofluorescent Staining of LDL Receptors

Perform all steps at room temperature and in the dark to maintain LDL-Dylight™ 550 staining. The following protocol is designed for a 96-well plate. For other sizes of plates the volume of medium/solution to apply to each well should be adjusted accordingly.

1. Remove most of the culture medium from the wells.
2. Wash cells with TBS, pH 7.4.
3. Fix the cells with 100 µl/well of Cell-Based Assay Fixative Solution (Item No. 10009899) for 10 minutes.
4. Wash the cells with TBS-Triton Buffer (prepared on page 6) three times for five minutes each.
5. Incubate the cells for 30 minutes with 100 µl/well of Cell-Based Assay Blocking Solution (Item No. 10009906).
6. Incubate the cells for one hour with 100 µl/well of diluted Rabbit Anti-LDL Receptor Primary Antibody.
7. Wash the cells with TBS-Triton Buffer three times for five minutes each.
8. Incubate the cells in the dark for one hour with 100 µl/well of diluted DyLight™ 488-Conjugated Secondary Antibody.
9. Wash the cells with TBS-Triton Buffer three times for five minutes each.
10. Examine the staining using a fluorescence microscope with a filter capable of excitation and emission at 485 and 535 nm, respectively. Alternatively, the plate may be stored in the dark at 4°C for later analysis. The staining should be stable for two to three days when plates are properly stored.

PERFORMANCE CHARACTERISTICS

Typical Cell Staining

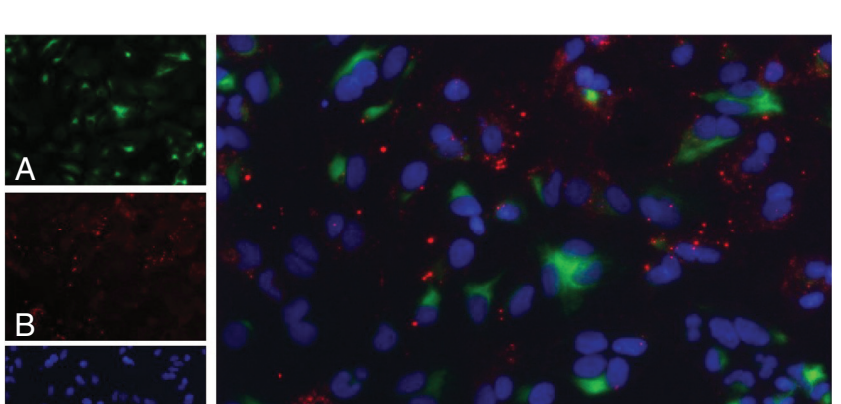

Figure 1: LDL uptake in Huh7 human hepatocytes. Huh7 cells were plated at 1 x 10^4 cells/well in a 96 well plate and allowed to attach and grow for 24 hours. LDL-Dylight™ 550 was added in serum-free medium at a dilution of 1:100 and incubated overnight. Nuclei were stained by addition of Hoechst 33342 (available as Item No. 15547) directly to the medium to a final concentration of 5 µM and incubation for 30 min at 37°C. LDLR staining was completed as described in the kit booklet. Fluorescent images (200X) were captured using a Nikon Eclipse microscope equipped with a Roper Scientific camera and MetaMorph software and merged in ImageJ. *Panel A:* LDLR staining in green, *Panel B:* LDL uptake in red, *Panel C:* Hoechst in blue, *Panel D:* a merged image.

Troubleshooting

Problem	Possible Causes	Recommended Solutions
No signal in all wells	A. Omission of key reagent B. Cells are not healthy	A. Check that all reagents have been added and in the correct order B. Use healthy cells
High signal in all wells	Over growth of cells	Make sure to plate cells at the correct density at the time of starting treatment

References

1. Soccio, R.E. and Breslow, J.L. Intracellular cholesterol transport. *Arterioscler. Thromb. Vasc. Biol.* **24**, 1150-1160 (2004).
2. Goldstein, J.L., Brown, M.S., Anderson, R.G.W., *et al*. Receptor-mediated endocytosis: Concepts emerging from the LDL receptor system. *Ann. Rev. Cell Biol.* **1**, 1-39 (1985).
3. Rudling, M.J., Reihnér, E., Einarsson, K., *et al*. Low density lipoprotein receptor-binding activity in human tissues: Quantitative importance of hepatic receptors and evidence for regulation of their expression *in vivo*. *Proc. Natl. Acad. Sci. USA* **87**, 3469-3473 (1990).

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.