LDL Uptake Cell-Based Assay Kit

Catalog Number KA1327
100 assays
Version: 07

Intended for research use only
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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.\(^3\)

Principle of the Assay

The 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\textsuperscript{TM} 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\textsuperscript{TM} 488-conjugated secondary antibody are included for identifying the distribution of LDL receptors.
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell-Based Assay Fixative</td>
<td>1 vial</td>
</tr>
<tr>
<td>Rabbit Anti-LDL Receptor Primary Antibody</td>
<td>1 vial</td>
</tr>
<tr>
<td>Cell-Based Assay Blocking Solution</td>
<td>1 vial</td>
</tr>
<tr>
<td>LDL-DyLight™ 550</td>
<td>1 vial</td>
</tr>
<tr>
<td>DyLight™ 488-Conjugated Goat Anti-Rabbit IgG Secondary Antibody</td>
<td>1 vial</td>
</tr>
</tbody>
</table>

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 us.

Storage Instruction

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

This kit will perform as specified if stored as described below and used before the expiration date indicated on the outside of the box.

<table>
<thead>
<tr>
<th>Component</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell-Based Assay Fixative</td>
<td>Room Temperature</td>
</tr>
<tr>
<td>Rabbit Anti-LDL Receptor Primary Antibody</td>
<td>-20°C</td>
</tr>
<tr>
<td>Cell-Based Assay Blocking Solution</td>
<td>4°C</td>
</tr>
<tr>
<td>LDL-DyLight™ 550</td>
<td>4°C</td>
</tr>
<tr>
<td>DyLight™ 488-Conjugated Goat Anti-Rabbit IgG Secondary Antibody</td>
<td>-20°C</td>
</tr>
</tbody>
</table>

Materials Required but Not Supplied

- Cells such as HepG2 (can be obtained from ATCC) and appropriate medium.
- A 0.45 μm syringe-top filter.
- TBS, pH 7.4
- Triton-X 100
- A fluorescence microscope equipped with filter sets capable of detecting fluorescein (excitation/emission = 485/535 nm) and rhodamine (excitation/emission = 540/570 nm)
Precautions for Use

✓ WARNING:
This product is for research use only. Not for human or veterinary diagnostic or therapeutic use.

✓ Precautions
Please read these instructions carefully before beginning this assay.

✓ Safety Data
The 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.
Assay Protocol

Reagent Preparation

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

1. LDL-DyLight™ 550 Working Solution - Dilute the LDL-DyLight™ 550 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 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 1:100 in TBS-Triton Buffer.

Assay Procedure

✔ 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 in below section.

✔ 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 for 10 minutes.
4. Wash the cells with TBS-Triton Buffer three times for five minutes each.

5. Incubate the cells for 30 minutes with 100 µL/well of Cell-Based Assay Blocking Solution.

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.
Data Analysis

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 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 protocol. 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.
Resources

Trouble shooting

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

References