

# **ab133127 – LDL Uptake Assay Kit (Cell-Based)**

## **Instructions for Use**

For the detection of LDL uptake into cultured cells.

This product is for research use only and is not intended for diagnostic use.



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## 1. Overview

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ab133127 provides a convenient tool for studying LDL uptake and regulation at the cellular level. The kit employs Human LDL conjugated to DyLight™ 550 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.

## 2. Background

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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. Regulation of cellular cholesterol metabolism and plasma cholesterol levels depends on low-density lipoprotein (LDL) receptor-mediated LDL uptake into specific cells.

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 extra-hepatic 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. On the other hand, activation of arterial endothelial cells increases transport of LDL into the vessel wall, causing the accumulation of cholesterol and formation of plaques which are characteristic of atherosclerosis. Studies have shown that modified LDL rather than native LDL is the major source of cholesterol accumulation in monocyte-derived macrophages responsible for foam cell formation. More recent work has demonstrated that native LDL is also taken up by macrophages and can also result in foam cell formation and development of plaques. Recent studies revealed abundant expression of the LDL receptor on murine macrophages, in contrast to previous reports of poor expression of these receptors on differentiated macrophages. The mechanism underlying the uptake of LDL by both hepatic tissue and cells of the cardiovascular system is not clearly understood. Thus, LDL uptake and its regulation are important therapeutic targets for atherosclerosis and related diseases.

### 3. Components and Storage

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| Item   | Quantity | Storage |
|--|----------|---------|
| <b>Cell-Based Assay Fixative</b>                                       | 1 vial   | RT      |
| <b>Rabbit Anti-LDL Receptor Primary Antibody</b>                       | 1 vial   | -20°C   |
| <b>Cell-Based Assay Blocking Solution</b>                              | 1 vial   | 4°C     |
| <b>LDL-DyLight™ 550</b>  | 1 vial   | 4°C     |
| <b>DyLight™ 488-Conjugated Goat Anti-Rabbit IgG Secondary Antibody</b> | 1 vial   | -20°C   |

#### Materials Needed But Not Supplied

- A 6-, 12-, 24-, or 96-well plate.
- HepG2 cells; other cell lines can also be used.
- Immunocytochemical staining buffer, 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).

## 4. Pre-Assay Preparation

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### Reagent Preparation

*NOTE: Reagents TBST, Rabbit Anti-LDL Receptor Primary Antibody and DyLight™ 488-Conjugated Goat Anti-Rabbit IgG Secondary Antibody should be prepared just prior to immunofluorescent staining of LDL receptors.*

#### **LDL-DyLight™ 550 Working Solution**

Dilute the LDL-DyLight™ 550 1:100 in serum-free cell medium. Filter through a 0.45 µM filter before adding to the cells.

#### **TBST**

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

#### **Rabbit Anti-LDL Receptor Primary Antibody**

Dilute the Rabbit Anti-LDL Receptor Primary 1:100 in TBST.

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

Dilute the DyLight™ 488-Conjugated Goat Anti-Rabbit IgG Secondary Antibody 1:100 in TBST.

## 5. Assay Protocol

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### A. Plate Configuration

There is no specific pattern for using the wells on the plate. A 6-, 12-, 24-, or a 96-well plate can be used. We recommend that each treatment is performed in triplicate.

#### *Pipetting Hints:*

- *It is recommended that an adjustable pipettor be used to deliver reagents to the wells.*
- *Before pipetting each reagent, equilibrate the pipette tip in that reagent (i.e., slowly fill the tip and gently expel the contents, repeat several times).*
- *Do not expose the pipette tip to the reagent(s) already in the well.*

### B. 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.

1. Seed a 96-well plate with  $3 \times 10^4$  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  $\mu$ l/well LDL-DyLight™ 550 working solution. Incubate the cells in a 37°C incubator for an additional three to four 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 immunocytochemical staining of LDL receptors using the procedure described below.

### **C. 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 briefly with TBS, pH 7.4.
3. Fix the cells with 100  $\mu$ l/well of Cell-Based Assay Fixative Solution for 10 minutes.
4. Wash the cells with TBST three times for five minutes each.
5. Incubate the cells for 30 minutes with 100  $\mu$ l/well of Cell-Based Assay Blocking Solution.
6. Incubate the cells for one hour with 100  $\mu$ l/well of dilute Rabbit Anti-LDL Receptor Primary Antibody.
7. Wash the cells with TBST three times for five minutes each.
8. Incubate the cells in the dark for one hour with 100  $\mu$ l/well of dilute DyLight<sup>TM</sup> 488-Conjugated Secondary Antibody.
9. Wash the cells with TBST 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.

## 6. Performance Characteristics

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### A. Cell Staining

Examples of typical staining of HepG2 cells (Figure 1) and adipocytes (Figure 2) obtained using this kit is shown below. Your results may vary based on the type of cell and the treatment used.

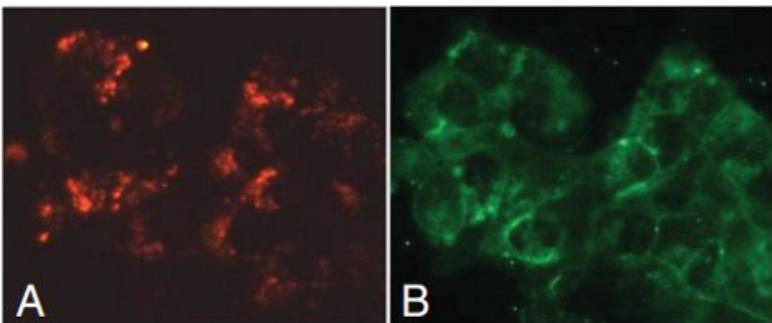


Figure 1: LDL uptake in HepG2 cells. HepG2 cells were cultured at a density of  $3 \times 10^4$  cells/well in a 96 well plate for two days then treated with  $32.5\mu\text{M}$  EGCG overnight. LDL-Dylight™ 550 ( $10\mu\text{g/ml}$ ) was added and the cells were incubated for an additional four hours. Cells were fixed and stained for LDL receptor using a Rabbit anti-LDL receptor primary antibody and Dylight™ 488-conjugated secondary antibody. Panel A: LDL-Dylight™ 550 taken into cells appears in red. Panel B: LDL receptors (in green) show a distribution pattern that matches the cells in the Panel A containing LDL-Dylight™ 550.

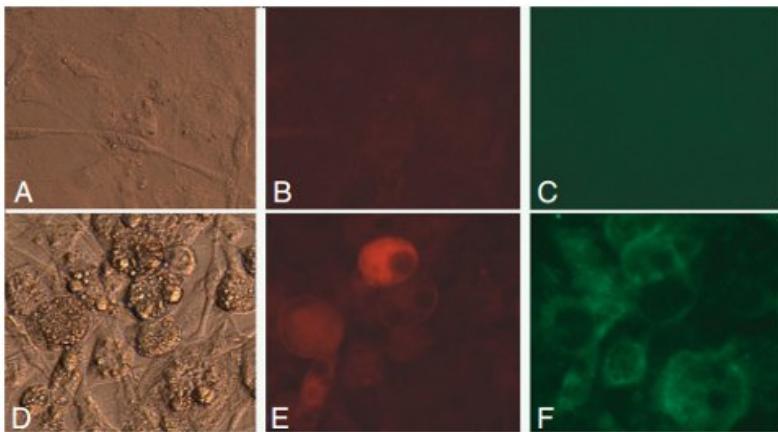


Figure 2: LDL Uptake in pre-adipocytes and adipocytes: 3T3-L1 cells were cultured at a density of  $3 \times 10^4$  cells/well in a 96-well plate d grown to confluence. The cells were then either maintained in the pre-adipocyte culture medium (upper Panels A-C) or cultured in differentiation induction medium (lower Panels D-F) for three days. On the fourth day, the culture medium in the control group was replaced with fresh medium and the culture medium in the differentiation group was changed to insulin medium. LDL-Dylight™ 550-conjugated LDL (10  $\mu$ g/ml) was added to the culture medium of both control and differentiation groups. Cells were cultured for another four days and then processed for LDL receptor immunostaining, according to the protocol described. Panel A: Light image of undifferentiated pre-adipocytes. Panel B: Undifferentiated pre-adipocytes do not show any LDL uptake. Panel C: Undifferentiated pre-adipocytes show little LDL receptor expression.

Panel D: Light image of differentiated adipocytes. Panel E: Corresponding cells to those in Panel D showing uptake of LDL (red). Panel F: Corresponding cells to those in Panel D showing expression of LDL receptor (green).

## 7. Troubleshooting

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





## Technical Support

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