Oxidized LDL Uptake Assay Kit

Item No. 601180

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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
601181	oxLDL-DyLight™ 488	1 vial/250 µl	4°C
601182	Cell-Based Assay Myricetin	1 vial/400 µg	4°C
400201	7-AAD Viability Dye (1,000X)	1 vial/50 µl	4°C
600332	Cell-Based Assay Hoechst Dye	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

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 oxLDL (*e.g.* THP-1 or RAW264.7 cell lines or primary macrophages), appropriate medium and cell dissociation reagents
2. DMSO
3. PBS, pH 7.4
4 Flow cytometer or fluorescence microscope equipped with lasers and filters capable of exciting and detecting the wavelengths shown in Table 1
5. A plate centrifuge
6. FACS tubes or v-bottom staining plates

INTRODUCTION

Background

The oxidation of low density lipoprotein is thought to be one of the first steps in atherosclerosis, the excessive buildup of inflammatory and cholesterol-rich plaques in the arteries. Oxidized LDL (oxLDL) may contribute to all stages of atherosclerosis by the activation of pro-inflammatory mediators that promote disease progression.[1] The initiation of LDL oxidation *in vivo* is uncertain, but circulating LDL can be oxidized by endothelial or inflammatory cells to play an important role in the formation of arterial plaques.[2] OxLDL is cytotoxic for endothelial cells, and stimulates their production of monocyte chemoattractants and maturation stimuli.[3] OxLDL is taken up by scavenger receptors (SRAI, SRAII and CD36) expressed by elicited monocytes and macrophages as well as local smooth muscle cells. The uptake of oxLDL by monocytes stimulates their invasion into plaque sites where further stimulation with oxLDL induces their transformation into lipid-laden foam cells.[4] Pro-atherogenic modifications of LDL include oxidation, acetylation, retention and aggregation, with oxidation being the most typical and physiological stimulus of macrophage foam cell formation.[5] The potential use of circulating oxLDL levels as a biomarker of cardiovascular disease progression as well as its corollaries, such as diabetes and obesity, highlights its centrality to the etiology of this family of diseases.[1]

About This Assay

Cayman's oxLDL Uptake Assay Kit employs human medium oxidized LDL conjugated to DyLight™ 488 as a convenient tool for studying modulators of the uptake of oxLDL in cultured cells. Myricetin, a flavonoid that has been reported to be an inhibitor of oxLDL uptake through CD36 transcriptional reduction in macrophages, is included as a control inhibitor.[6] The reagents provided in this kit are sufficient to run a single plate of cultured cells by either flow cytometry or fluorescence microscopy.

Reagent	Ex/Em Filters	Flow cytometric laser/filter sets	What it detects
DyLight™ 488	493/518	488/525	Cells that have taken up oxLDL
7-AAD	546/647	488/655-730	Nuclei of dead cells only (plasma membrane permeability)
Hoechst Dye	350/461	350 (or 405)/450	All nuclei

Table 1. Dye combination included in this kit and optimal detection methods for fluorescence microscopy and flow cytometry

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Myricetin Solution**

   Reconstitute the Cell-Based Assay Myricetin (Item No. 601182) with 50 µl of DMSO to make a 25 mM solution. Mix well. After use, this Myricetin Solution can be stored at -20°C and should be stable for up to 6 months.

2. **7-AAD Staining Solution**

   Add 10 µl of Cell-Based Assay 7-AAD Staining Stock Solution (1,000X) (Item No. 400201) to 10 ml of PBS and mix well. This solution should be used immediately.

3. **Hoechst Staining Solution**

   Add 5 µl of Cell-Based Assay Hoechst Dye (Item No. 600332) to 10 ml of PBS and mix well. This solution should be used immediately.

ASSAY PROTOCOL

Flow cytometry

NOTE: It is recommended for flow cytometric applications to prepare single-stained control samples for the purposes of fluorescence compensation.

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. To use the Myricetin Solution prepared on page 7 as an inhibitor of oxLDL uptake, dilute to a final concentration of 10-100 µM in culture medium at least 2 hours prior to addition of oxLDL-DyLight™ 488.
2. Prior to the end of the treatment period, add oxLDL-DyLight™ 488 (Item No. 601181) to a final, in-well dilution of 1:50. Incubate the cells at 37°C for an additional 4-24 hours, or for the period of time used in your typical experimental protocol.
3. Collect cells into FACS tubes (or alternatively a 96 well, v-bottom polypropylene plate) and centrifuge for 5 minutes at 250 x g. oxLDL will stimulate the adherence of some cell lines, in which case scraping may be necessary to remove the cells from the treatment plate.
4. Wash once with 200–500 µl PBS, pH 7.4.
5. Centrifuge at 250x g for five minutes. Carefully remove the supernatant.
6. *Optional: Stain surface markers as required using a standard staining protocol.*
7. Resuspend the cells in 100 µl of 7-AAD Staining Solution per 100,000 cells. Mix well to ensure separation of individual cells.
8. Analyze the cells with a flow cytometer using the channels shown in Table 1, on page 6. The cells must be analyzed immediately.

Fluorescence Microscopy

NOTE: This protocol is designed for use in a 24-well plate or 4-well chamber slide, 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. To use the included Myricetin Solution prepared on page 7 as an inhibitor of oxLDL uptake, dilute to a final concentration of 10-100 µM in culture medium at least 2 hours prior to addition of oxLDL-DyLight™ 488.
2. Prior to the end of the treatment period, add oxLDL-DyLight™ 488 (Item No. 601181) to a final, in-well dilution of 1:50. Incubate the cells at 37°C for an additional 4-24 hours, or for the period of time used in your typical experimental protocol.
3. Remove culture medium from each well, being careful not to disturb the cell layer.
4. Add 500 µl of the Hoechst Staining Solution prepared on page 7 to each well of the plate.
5. Incubate samples at 37°C in the dark for 15 minutes.
6. Visualize cells on your fluorescence microscope using the filter sets shown in Table 1, on page 6.

ANALYSIS

Performance Characteristics

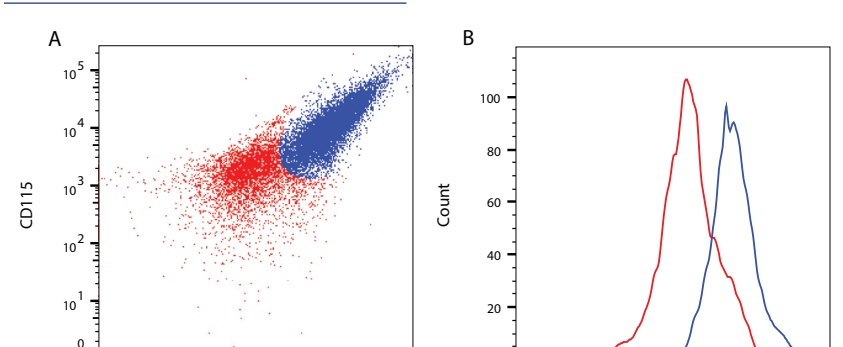

Figure 1: Thioglycolate-elicited peritoneal macrophages take up oxLDL. Three female BALB/c mice were injected i.p. with 1 ml of thioglycolate. After 72 hours, the mice were euthanized and the peritoneal cavity was lavaged with 5 ml of RPMI + 10% FBS. The collected cells were counted and plated at a density of 250,000 cells per well in a 24-well plate. Cells were incubated with the oxLDL-DyLight™ 488 for 16 hours before staining with CD11b and CD115 surface markers followed by 7-AAD staining. Data were collected by a flow cytometer and analyzed in FlowJo by Treestar Inc. For analysis, cells were divided into CD11b+/CD115+ peritoneal macrophages (blue) and other cells (red) (*Panel A*) and visualized for oxLDL-DyLight™ 488 within the 7-AAD negative population (*Panel B*).

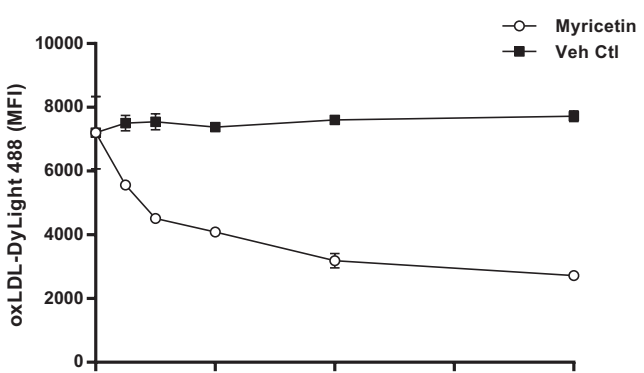

Figure 2: Myricetin inhibits oxLDL uptake in RAW264.7 cells. RAW264.7 cells were plated at a density of 250,000 cells per well in a 24-well plate and allowed to attach overnight. The following day, the medium was replaced with DMEM + 10% FBS containing Myricetin at the concentrations indicated for 2 hours before adding oxLDL-DyLight™ 488 at a final dilution of 1:50 for 4 hours. Cells were then processed according to the Flow Cytometry protocol in this kit booklet. Cells were gated on 7-AAD negative events, and the geometric mean fluorescence intensity (MFI) of oxLDL was determined and graphed.

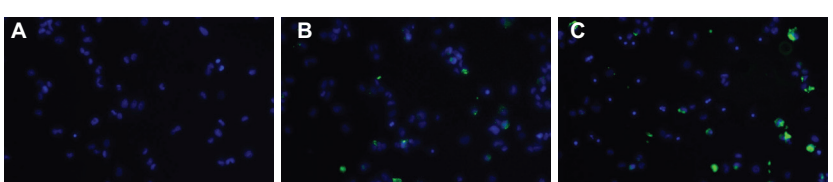

Figure 3. Uptake of oxLDL in THP-1 derived macrophages. THP-1 cells were differentiated into macrophages by treating with 200 nM phorbol 12-myristate 13-acetate (PMA) for 72 hours. After differentiation, oxLDL-DyLight™ 488 was added at either a dilution of 1:50 (*Panel B*), 1:10 (*Panel C*) or no oxLDL-DyLight™ 488 (*Panel A*) for 4 hours. Cells were then processed according to the Fluorescence Microscopy protocol in this kit booklet and imaged on a fluorescence microscope at 200X magnification. Hoechst-stained nuclei are shown in blue and oxLDL is shown in green.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Strong 7-AAD staining in all samples, including controls	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.
No cells seen	Cells lost during processing	A. Decrease treatment time or compound dosage B. Wash and process cells more gently
Little or no oxLDL-DyLight™ 488 signal	A. Too short of an uptake time for the cell type used B. Cells being used do not take up oxLDL C. Incorrect filters used to visualize oxLDL	A. Optimize uptake time for each cell type used B. Ensure cell line is used that will take up oxLDL C. Use filters indicated in Table 1, on page 6
Replicates have varying values	A. oxLDL aggregates B. oxLDL sticks to side of cell culture vessels	A. Filter oxLDL solution through 0.45 µM filter before adding to cells B. Avoid vortexing or vigorous mixing C. Add oxLDL solution to media directly, not to vessel wall

References

1. Trpkovic, A., Resanovic, I., Stanimirovic, J., *et al.* Oxidized low-density lipoprotein as a biomarker of cardiovascular diseases. *Crit. Rev. Clin. Lab. Sci.* **52(2)**, 70-85 (2015).
2. Steinberg, D. Low density lipoprotein oxidation and its pathobiological significance. *J. Biol. Chem.* **272(34)**, 20963-20966 (1997).
3. Rajavashisth, T.B., Andalibi, A., Territo, M.C., *et al.* Induction of endothelial cell expression of granulocyte and macrophage colony-stimulating factors by modified low-density lipoproteins. *Nature* **344(6263)**, 254-257 (1990).
4. Park, Y.M. CD36, a scavenger receptor implicated in atherosclerosis. *Exp. Mol. Med.* **46**, e99 (2014).
5. Xu, S., Huang, Y., Xie, Y., *et al.* Evaluation of foam cell formation in cultured macrophages: An improved method with Oil Red O staining and DiI-oxLDL uptake. *Cytotechnology* **62(5)**, 473-481 (2010).
6. Lian, T.-W., Wang, L., Lo, Y.-H., *et al.* Fisetin, morin and myricetin attenuate CD36 expression and oxLDL uptake in U937-derived macrophages. *Biochim. Biophys. Acta* **1781(10)**, 601-609 (2008).

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.