Version 1 Last updated 4 April 2019

ab236208
LDL Uptake Assay Kit (Flow cytometry)

For the measurement of cellular LDL uptake in cell cultures.

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

Table of Contents

1. Overview

The LDL Uptake Assay Kit (Flow cytometry) (ab236208) 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, it is possible to gain more information from a single experiment.

The reagents provided in this kit are sufficient to test 48 samples by flow cytometry.

Prepare reagents and cells.

Dilute lovastatin to 1 µM and treat cells for 24 hours.

Four hours before the end of the treatment, add LDL-Dylight™ 488 and incubate at 37°C for four hours in the dark.

Centrifuge cells at 250 x *g* for 5 minutes. Resuspend cells and centrifuge again.

Resuspend cells in 7-AAD Dye.

Collect data detecting LDL-Dylight™ 488 in the FITC channel and 7-AAD in the PE or PI/PerCP channel.

2. Materials Supplied and Storage

Store kit at 4°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
LDL-DyLight™ 488 Assay Reagent	120 µL	4°C	Do not store
Lovastatin Control	0.1 mg	4°C	-20°C
Cell-Based Assay Buffer Tablet	1 tablet	RT	RT
Cell-Based Assay 7-AAD Staining Stock Solution (1000X)	50 µL	4°C	Do not store

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

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

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 LDL-DyLight™ 488 Assay Reagent

1. Dilute LDL-DyLight™ 488 Assay Reagent 1:10 in your culture medium. Dilute immediately before use.
2. If particulates are observed, filter diluted LDL-DyLight™ 488 through a 0.45 µm syringe-top filter.

ΔNote: Do not vortex.

5.2 Lovastatin Control

1. Resuspend Lovastatin control in 250 µL DMSO.

ΔNote: This stock is a 1 mM solution of lovastatin, which should be stored after use at -20°C for up to 6 months.

5.3 Cell-Based Assay Buffer Tablet

1. Dissolve tablet in 100 mL of distilled water.

1. **Cell-Based Assay 7-AAD Staining Stock Solution (1000X)**

   Dilute the Cell-Based Assay 7-AAD Staining Stock Solution by adding 10 µL into 10 mL of Cell-Based Assay Buffer. This solution should be used immediately.

6. Assay Procedure

Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.

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

6.1 Flow cytometry

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. The 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 in total with Lovastatin to increase LDL uptake.
3. Four hours before the end of the treatment, add 25 µL diluted LDL-Dylight™ 488 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 Cell-Based Assay Buffer.
7. Centrifuge cells at 250 x *g* for five minutes and remove supernatant.
8. Resuspend cells in 100-200 µL diluted Cell-Based Assay 7-AAD Staining Stock Solution, triturating gently to obtain a single cell suspension. The compensation control cells (step 6.1.4) should be resuspended in Cell-Based 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.

ΔNote: Some cell types which are ideal for LDL uptake (e.g. HepG2) can be difficult to make into a single-cell suspension, so exclusion of doublets may be necessary for generating robust data.

7. FAQs / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

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

8. Typical Data

Data provided **for demonstration purposes only.**

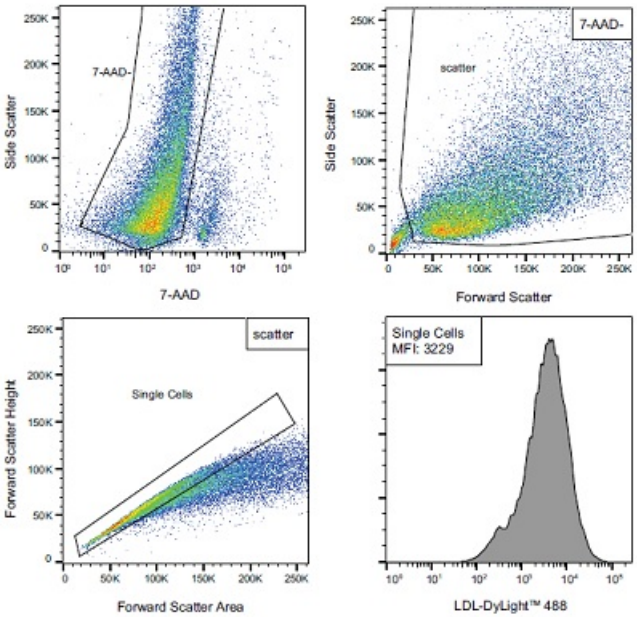

Figure 1. Example flow cytometric analysis. HepG2 cells were plated at 2 x 10^5 cells/well in a 24 well plate approximately 48 hours before addition of LDL-DyLight™ 488. After a four hour incubation with the LDL probe, cells were trypsinized, washed and stained with 7-AAD prior to flow cytometry. In 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.

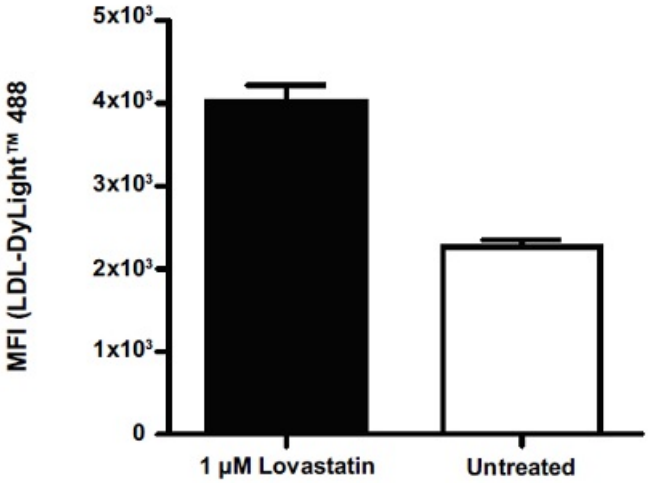

Figure 2. Lovastatin increases uptake of LDL. HepG2 cells were plated at 2 x 10^5 cells/well in a 24 well plate and allowed to adhere overnight, before being treated with 1 µM Lovastatin or left untreated for 24 hours in MEM + 2% FBS. The final four hours of treatment included the probe LDL-DyLight™ 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 intestities (MFI) for each group are plotted.

9. Notes

Technical Support

Austria
wissenschaftlicherdienst@abcam.com | 019-288-259
France
supportscientifique@abcam.com | 01.46.94.62.96
Germany
wissenschaftlicherdienst@abcam.com | 030-896-779-154
Spain
soportecientifico@abcam.com | 91-114-65-60

Switzerland
technical@abcam.com
Deutsch: 043-501-64-24 | Français: 061-500-05-30
UK, EU and ROW
technical@abcam.com | +44(0)1223-696000

Canada
ca.technical@abcam.com | 877-749-8807
US and Latin America
us.technical@abcam.com | 888-772-2226

Asia Pacific
hk.technical@abcam.com | (852) 2603-6823
China
cn.technical@abcam.com | 400 921 0189 / +86 21 2070 0500
Japan
technical@abcam.co.jp | +81-(0)3-6231-0940
Singapore
sg.technical@abcam.com | 800 188-5244

Australia
au.technical@abcam.com | +61-(0)3-8652-1450
New Zealand
nz.technical@abc.com | +64-(0)9-909-7829