Low-Density Lipoproteins And Their Conjugates

Pub. No. MAN0001842 **Rev.** A.0

Table 1. LDL stand-alone reagents

LDL complex	Modification	Cat. No.	Ex/Em[1]	Content	Storage[2]
Unlabeled LDL	Native (unoxidized)	L3486	N/A	200 µL (2.5 mg/mL) LDL	• 2–8°C • Do not freeze • Protect from light • Protect from air
BODIPY™ FL LDL[3]		L3483	515/520	200 µL (1 mg/mL) LDL	
DiI LDL[3]		L3482	554/571	200 µL (1 mg/mL) LDL	
pHrodo™ Green LDL		L34355	509/533	200 µL (1 mg/mL) LDL	
pHrodo™ Red LDL		L34356	560/585	200 µL (1 mg/mL) LDL	
Ox-LDL	Oxidized	L34357	N/A	200 µL (2.5 mg/mL) LDL	
DiI Ox-LDL		L34358	554/571	200 µL (1 mg/mL) LDL	
Unlabeled AcLDL	Acetylated	L35354	NA	200 µL (2.5 mg/mL) LDL	
Alexa Fluor™ 488 AcLDL		L23380	495/519	200 µL (1 mg/mL) LDL	
DiI AcLDL[3]		L3484	554/571	200 µL (1 mg/mL) LDL	
Alexa Fluor™ 594 AcLDL		L35353	590/617	200 µL (1 mg/mL) LDL	

[1] Approximate fluorescence excitation and emission maxima, in nm.

[2] When stored as directed, LDL products are stable for four to six weeks from the date of shipment. AcLDL products are stable for two to three months from the date of shipment.

[3] This fluorescent label is not covalently attached to the complex; therefore, the product may not be suitable for applications that require cell fixation before analysis.

N/A: Not applicable.

Table 2. Native LDL uptake kits

LDL complex	Cat. No.	Ex/Em[1]	Content	Storage[2]
BODIPY™ FL LDL Uptake Kit	I34359	515/520	Labeled LDL, 200 µL (1 mg/mL) (Component A)	• 2–8°C • Do not freeze • Protect from light • Protect from air
			Unlabeled LDL, 200 µL (2.5 mg/mL) (Component B)	
			1X LDL Assay Buffer, 50 mL (Component C)	
			Heparin, 200 µL (5 mg/mL) (Component D)	
			BSA (Blocker), 150 mg (Component E)	
pHrodo™ Red LDL Uptake Kit	I34360	560/585	Labeled LDL, 200 µL (1 mg/mL) (Component A)	• 2–8°C • Do not freeze • Protect from light • Protect from air
			Unlabeled LDL, 200 µL (2.5 mg/mL) (Component B)	
			1X LDL Assay Buffer, 50 mL (Component C)	
			Heparin, 200 µL (5 mg/mL) (Component D)	
			BSA (Blocker), 150 mg (Component E)	

[1] Approximate fluorescence excitation and emission maxima, in nm.

[2] When stored as directed, LDL products are stable for four to six weeks from the date of shipment.

N/A: Not applicable.

Product description

Low-density lipoprotein (LDL) complexes

Human low-density lipoprotein (LDL) is one of the key lipid-protein complexes in the blood and is a crucial component of metabolism responsible for the transport of lipids throughout the body. LDL is composed primarily of cholesterol, cholesterol esters, triglycerides, phospholipids, and a single Apo B-100 protein. LDL delivers fatty acids to peripheral cells through the action of lipoprotein lipase and cholesterol and fatty acids through receptor-mediated endocytosis. While absolutely essential to meet the body's energy demands, LDL can also be detrimental to health. Aberrant regulation of LDL blood concentration and/or oxidation supports the formation of atherosclerotic plaques, which is a key underlying factor in the development of many cardiovascular diseases.

We offer an array of human LDL particles (Table 1, page 1) to aid in the understanding of how LDL interacts with different cell and tissue types . Labeled LDL can be used to study LDL uptake through endocytosis and its trafficking throughout the cell. Our fluorescently labeled LDLs have been designed for use with fluorescence microscopy, flow cytometry, cell sorting, high-throughput screening, and high-content analysis.

Native (unmodified) LDL

We offer native LDL with traditional labels such as BODIPY™ FL and DiI as well as with the pH-sensitive labels pHrodo™ Green and pHrodo™ Red (Table 1, page 1). The pHrodo™ line of dyes offer extra functionality, specificity, and ease of use. Because these dyes only fluoresce once they are inside the cell, they eliminate the need for washing away excess LDL or removing the LDL containing media. This allows real-time kinetic measurements of LDL uptake and trafficking, as they follow the acidification of LDL containing endosomes during maturation. While live sample dynamic studies are possible with pHrodo™ Red and pHrodo™ Green-labeled LDL, single time point measurements can be achieved with formaldehyde-fixable BODIPY™ FL and DiI.

Native LDL is isolated from human plasma, which is sourced from a blood bank and tested for HIV, hepatitis B and C, syphilis, and other infectious diseases. Each lot of LDL is tested for oxidation and extensively purified to be free of HDL and other contaminating lipoproteins before labeling or other modifications.

Oxidized LDL

Oxidation of LDL is a natural process within the body, and oxidized LDL complexes are an important tool for the study of scavenger receptor-mediated endocytosis by macrophages and endothelial cells, and of the formation of foam cells.

To prepare oxidized LDL, native LDL is oxidized by a copper-mediated process to the optimal degree of oxidation. After testing the degree of oxidation, each lot is functionally tested with Bovine Pulmonary Artery Epithelium (BPAE) cells for their ability to be recognized by scavenger receptors (Figure 5, page 5).

Acetylated LDL (AcLDL)

Acetylation of the lysine residues of B-100 is a classic approach for the study of specific scavenger receptors on the surface of macrophages and endothelial cells and of the formation of foam cells.

LDL uptake kits

Our LDL uptake kits (Table 2, page 1) include labeled LDL, unlabeled LDL, heparin, BSA, and assay buffer. They are designed to specifically detect binding and uptake of LDL through the LDL receptor internalization pathway, allowing maximum control and flexibility in experimental design. Two controls are built into the system, unlabeled LDL and heparin. Unlabeled LDL provides an optional pre-treatment to occlude cell surface receptors in advance of probing with the labeled construct, while heparin is provided as an additional control to chelate the LDL in the solution phase outside of the cells, thus preventing binding and uptake of the labeled LDL. Figures 2,3, and 4 (page 4) indicate that the uptake of LDL without treatment is specifically mediated with LDL receptors.

We offer two varieties of LDL uptake kits, one that includes the traditional LDL label BODIPY™ FL, and the other the pH-sensitive label pHrodo™ Red (Table 2, page 1). pHrodo™ Red-labeled LDL particles are dimly fluorescent at neutral pH outside of cells, but fluoresce brightly after uptake. Because of its activation at acidic pH, pHrodo™ Red-labeled LDL allows for kinetic measurement of uptake and endosomal maturation without the need for extensive washing, allowing easy discrimination of intracellular LDL from cell surface bound LDL and providing greater specificity.

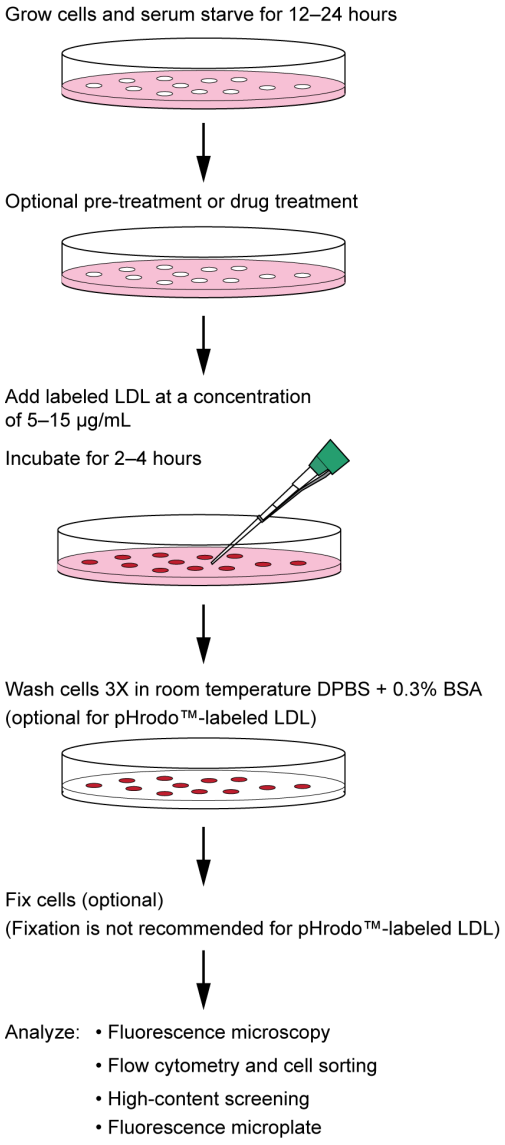

Figure 1. Typical labeling and analysis workflow using labeled LDLs.

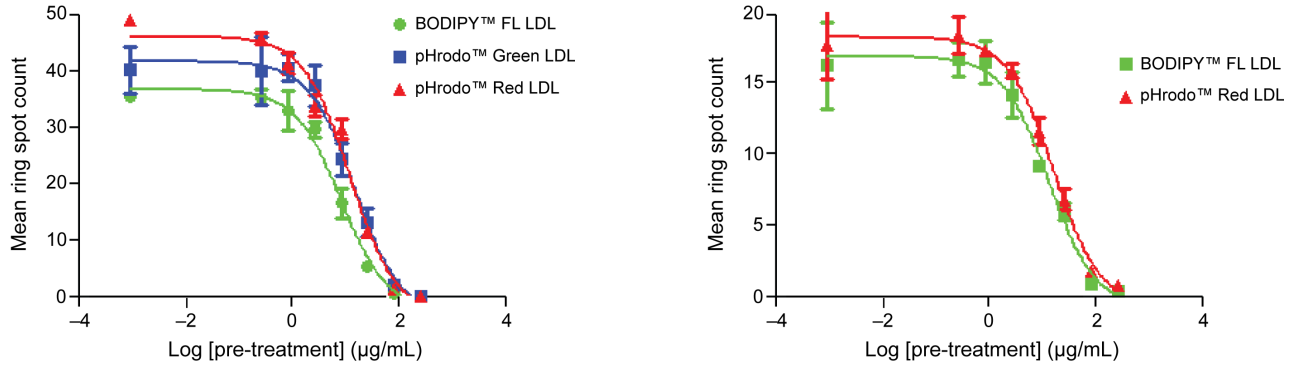

Figure 2. HEPG2 cells were plated in Poly-D-Lysine-coated 96-well plates for 24 hours, then serum starved overnight in Fluorobrite™ DMEM medium (Cat. No. A1896702) plus 0.3% BSA. On the day of the experiment, cells were pre-treated with a dilution series of unlabeled LDL (Cat. No. L3486) from 250 µg/mL to zero (left panel) or Heparin from 500 µg/mL to zero (right panel) for 30 minutes at 37°C, then probed with 5 µg/mL of BODIPY™ FL LDL (Cat. No. L3483) (green), with 10 µg/mL of pHrodo™ Green LDL (Cat. No. L34355) (blue), or with pHrodo™ Red LDL (Cat. No. L34356) (red) for three hours in the cell culture incubator. BODIPY™ FL LDL-treated wells were rinsed with Assay Buffer plus 0.3% BSA and fixed in 4% PFA before imaging, while the pHrodo™ LDL-treated wells were imaged live after nuclear staining with Hoechst dye. Images were acquired and enumerated on the CellInsight™ CX5 HCS platform. Data analysis was performed with the internalization and spot count module in the Studio™ Cell Analysis software to quantify the number of label-positive spots per cell. 500 cells were sampled per well, with n=4 wells per data point.

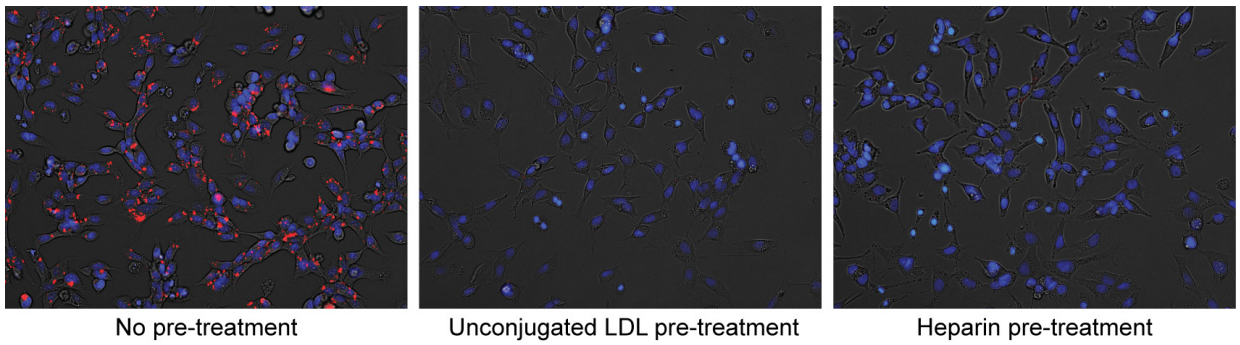

Figure 3. HEPG2 cells were plated onto Poly-D-Lysine-coated glass bottom dishes in growth medium and allowed to recover for 24 hours before switching to starvation medium overnight, using Fluorobrite™ DMEM medium (Cat. No. A1896702) plus 0.3% BSA. On the day of imaging, cells were pre-treated with either vehicular control solution (left panel), unlabeled LDL at 250 µg/mL (Cat. No. L3486) (center panel) or Heparin at 250 µg/mL (right panel) for 30 minutes at 37°C in the cell culture incubator. pHrodo™ Red LDL (Cat. No. L34356) (pseudocolor red) was added to the medium at 10 µg/mL and the cells were returned to the incubator for 3 hours. NucBlue™ Live nuclear stain (Cat. No. R37605) (pseudocolor blue) was added to the cultures at 2 drops per mL concentration at 37°C in last 30 minutes. Cells were then washed twice in Assay Buffer solution (HBSS plus 3% BSA) and the images were captured on the EVOS™ FL Auto 2 Imaging System. Data shown was overlaid with white light, RFP light cube for pHrodo™ Red LDL (pseudocolor red) and DAPI light cube for NucBlue™ Live stain (pseudocolor blue).

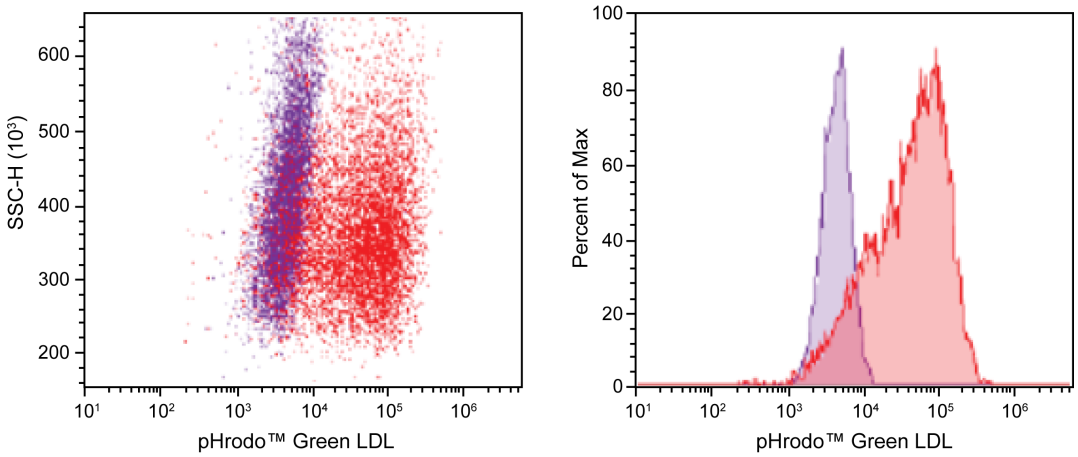

Figure 4. THP-1 cells were serum starved overnight in RPMI 1640 medium plus 0.3% BSA. On the day of analysis, cells were pre-treated with either vehicular control solution (red) or unlabeled LDL (Cat. No. L3486) (purple) center panel at 250 µg/mL for minutes at 37°C in the cell culture incubator. pHrodo™ Green LDL (Cat. No. L34355) was added to the medium at 10 µg/mL and the cells were returned to the incubator for 3 hours. Flow cytometric analysis was performed using the Attune™ NxT Flow Cytometer BHL-1 channel. Vehicular control shows 10 times more fluorescence than when pre-treatment with unlabeled LDL occludes cellular entry of pHrodo™ Green LDL.

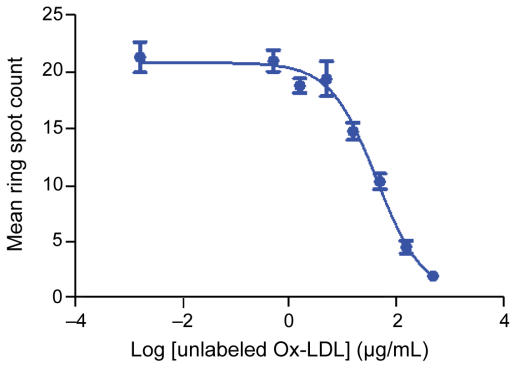

Figure 5. Pre-treatment with unlabeled Ox-LDL occludes entry of DiI-labeled, Ox-LDL in a specific and dose-dependent manner. BPAE cells were plated in Poly-D-Lysine coated 96-well plates for 24 hours, then serum starved overnight in Fluorobrite™ DMEM medium (Cat. No. A1896702) plus 0.3% BSA. On the day of the experiment, cells were pre-treated with a dilution series of unlabeled, Ox-LDL from 500 µg/mL to zero for 30 minutes at 37°C, then probed with 10 µg/mL of DiI Ox-LDL for three hours in the cell culture incubator. Cells were rinsed with Assay Buffer plus 0.3% BSA and fixed in 4% PFA. Following nuclear staining with Hoechst dye, images were acquired and enumerated on the CellInsight™ CX5 HCS platform. Data analysis was performed with the internalization and spot count module in the Studio™ Cell Analysis software to quantify the number of label-positive spots per cell. 500 cells were sampled per well, with n=3 wells per data point.

Materials

Materials not provided, but may be needed

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Cat. No.
Hep G2 [HEPG2] Cells	ATCC™, HB-8065
Bovine Pulmonary Artery Endothelial Cells (BPAEC) Cells	Cell Applications, Inc, B302-05
HBSS, calcium, magnesium, no phenol red	14025126
DPBS, calcium, magnesium	14040133
Immunoassay Grade, Ultra Pure BSA	MLS
FluoroBrite™ DMEM	A1896702
NucBlue™ Live ReadyProbes™ Reagent	R37605
Image-iT™ Fixative Solution (4% formaldehyde, methanol-free)	FB002
ProLong™ Diamond Antifade Mountant	P36961

Native LDL, OxLDL, AcLDL

Unlabeled native LDL, OxLDL, and AcLDL are supplied in units of 200 µL at a concentration of 2.5 mg/mL in 10 mM Tris, 150 mM NaCl, 0.3 mM EDTA, pH 8.3, containing 2 mM sodium azide to inhibit bacterial contamination.

The labeled native LDL, OxLDL, and AcLDL complexes and conjugates are supplied in units of 200 µL at a concentration of 1 mg/mL in the same buffer as the unlabeled LDL.

Working Assay/Wash Buffer

When using LDL uptake kits, mix 150 mg of BSA (Component E) with 1X LDL Assay Buffer (Component B), which is composed of HBSS (Cat. No. 14025126).

Alternatively, you can also use DPBS with calcium and magnesium (Cat. No. 14040133) as LDL Assay Buffer. When using DPBS or an alternative buffer, mix 50 mL of DPBS with 150 mg of high quality BSA, to preprare the Working Assay/Wash Buffer

You can use LDL Assay Buffer for the wash steps described in the protocol below.

Note: The binding of LDL to the LDL receptor requires the presence of Ca^{2+} and Mg^{2+} and is inhibited by excess EDTA.

When using pHrodo™ Green or Red labeled LDL, the wash steps are optional. If you wish to load and image LDL in complete medium, prepare complete medium using FluoroBrite™ DMEM (Cat. No. A1896702) to reduce the background fluorescence.

Note: When using pHrodo™ labeled LDL reagents, further adjustment of extracellular pH with NaOH to 7.5 or higher will suppress extracellular fluorescence and enhance the contrast of internalized LDL.

Methods

The following protocol is optimized for HepG2 cells for native LDL uptake, and for BPAEC cells for OxLDL or AcLDL uptake. For other cell types, the protocol may need to be altered for optimal results.

1. Grow the cells to 30–40% confluence.
2. To serum starve the cells, prepare media without serum, but with 0.3% BSA. If your cell type requires the use of serum, then use serum which has been depleted for LDL.

   IMPORTANT! If you are using pHrodo™ Red or Green labeled LDL and do not wish to perform the optional wash step, we recommend that you prepare serum starvation medium with FluoroBrite™ DMEM plus 0.3% BSA.
3. Rinse the cells once with serum starvation medium, then incubate the cells in serum starved medium for 12–24 hours.
4. *Optional:* At the end of the incubation, rinse the cells once with Working Assay/Wash Buffer (page 6). Otherwise, proceed with cells grown in serum starvation medium.
5. *Optional:* To specifically block the LDL receptor uptake, we recommend pre-treatment with unlabeled LDL to occlude the LDL receptors or with Heparin to bind extracellular LDL.

   To pre-treat the cells, dilute the unlabeled LDL or Heparin 1:10 into the cells and incubate for 30–60 minutes before adding the labeled probe. We recommend working concentrations of 250 µg/mL of unlabeled LDL or 500 µg/mL of Heparin for pre-treatment.
6. Dilute labeled LDL in Working Assay/Wash Buffer to 50 µg/mL or 100 µg/mL for a 10X solution. Add this solution to the medium used for the previous cell incubation at 1X concentration. For most cell types 5–15 µg/mL of working concentration works well; for others cell types this concentration may need to be optimized.

7. Incubate the cells with the labeled LDL solution for 2–4 hours at 37°C.

   Note: If you are performing the assay in serum starvation medium, make sure to return the cells to the incubator for proper pH buffering.

8. Remove the cells from the 37°C incubation and rinse 2–3X with Working Assay/Wash Buffer at room temperature. This step is optional for pHrodo™ Red or Green labeled LDL.

9. For optional nuclear stain, add 2 drops of NucBlue™ Live ReadyProbes™ Reagent per 1 mL of Working Assay/Wash Buffer, then incubate cells for 30 minutes at room temperature.

   Note: Alternatively, you can combine this step with Step 7 and add the NucBlue™ Live ReadyProbes™ Reagent at 2 drops per mL to the labeled LDL in the last 30 minutes of the incubation.

10. *Optional:* To fix DiI LDL or BODIPY™ FL LDL-labeled cells, incubate the cells at room temperature for 15–30 minutes with Image-iT™ Fixative Solution (4% formaldehyde, methanol-free) or freshly prepared 4% formaldehyde solution in DPBS.

    Note: Fixation with formaldehyde is not recommended for pHrodo™ Red LDL and pHrodo™ Green LDL.

11. *Optional:* For high resolution images, cells labeled with DiI LDL or BODIPY™ FL LDL can be mounted with ProLong™ Diamond Antifade Mountant when using glass plate or slides.

12. Analyze the cells with fluorescent microscope, flow cytometer, fluorescent microplate reader, or high content imager.

Ordering information

Cat. no.	Product Name	Unit Size
I34359	Image-iT™ Low Density Lipoprotein Uptake Kit - BODIPY™ FL	1 kit
I34360	Image-iT™ Low Density Lipoprotein Uptake Kit - pHrodo™ Red	1 kit
L34356	Low Density Lipoprotein From Human Plasma, pHrodo™ Red (pHrodo™ Red-LDL) *1 mg/mL*	200 µL
L34355	Low Density Lipoprotein From Human Plasma, pHrodo™ Green (pHrodo™ Green-LDL) *1 mg/mL*	200 µL
L3483	Low Density Lipoprotein From Human Plasma, BODIPY™ FL complex (BODIPY™ FL LDL) *1 mg/mL*	200 µL
L3482	Low Density Lipoprotein From Human Plasma, DiI complex (DiI LDL) *1 mg/mL*	200 µL
L3486	Low Density Lipoprotein From Human Plasma (LDL) *2.5 mg/mL*	200 µL
L34357	Low Density Lipoprotein From Human Plasma, Oxidized (OxLDL) *2.5 mg/mL*	200 µL
L34358	Low Density Lipoprotein From Human Plasma, Oxidized, DiI complex (DiI-OxLDL) *1 mg/mL*	200 µL
L23380	Low Density Lipoprotein From Human Plasma, Acetylated, Alexa Fluor™ 488 conjugate (Alexa Fluor™ 488 AcLDL) *1 mg/mL*	200 µL
L35353	Low Density Lipoprotein From Human Plasma, Acetylated, Alexa Fluor™ 594 conjugate (Alexa Fluor™ 594 AcLDL) *1 mg/mL*	200 µL
L3484	Low Density Lipoprotein From Human Plasma, Acetylated, DiI complex (DiI-AcLDL) *1 mg/mL*	200 µL
L35354	Low Density Lipoprotein From Human Plasma, Acetylated, (AcLDL) *2.5 mg/mL*	200 µL
Related Products		
14025126	HBSS, calcium, magnesium, no phenol red	6 × 1000 mL
14040133	DPBS, calcium, magnesium	500 mL
A1896702	FluoroBrite™ DMEM	10 × 500 mL
FB002	Image-iT™ Fixative Solution (4% formaldehyde, methanol-free)	20 mL
P36961	ProLong™ Diamond Antifade Mountant	5 × 2 mL
R37605	NucBlue™ Live ReadyProbes™ Reagent	1 kit

Purchaser notification

These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

Obtaining support
For the latest services and support information for all locations, go to **thermofisher.com/support**.

At the website, you can:
- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**thermofisher.com/support**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

SDS
Safety Data Sheets (SDSs) are available at **thermofisher.com/support**.

Certificate of Analysis
The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **thermofisher.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the product packaging (tube, pouch, or box).

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Revision history: Pub. No. MAN0001842

Revision	Date	Description
A.0	January 2017	Add new products and include methods chapter, revise Product information
1.0	July 2006	Basis for this revision

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