

Low-Density Lipoproteins

Quick Facts

Storage upon receipt:

- 2–6°C
- Do not freeze
- Protect from light
- Protect from air

Abs/Em: See Table 1

Note: 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.

Table 1. Molecular Probes' LDL products and their spectral characteristics.

Cat#	LDL Complex	Abs *	Em *
LDL Products			
L3486	Unlabeled LDL	NA	NA
L3483	BODIPY® FL LDL †	515	520
L3482	DiI LDL †	554	571
Acetylated LDL Products			
L35354	Unlabeled AcLDL	NA	NA
L23380	Alexa Fluor® 488 AcLDL	495	519
L3485	BODIPY® FL AcLDL	510	518
L3484	DiI AcLDL †	554	571
L35353	Alexa Fluor® 594 AcLDL	590	617

* Approximate absorption (Abs) and fluorescence emission (Em) maxima, in nm. † 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. NA = not applicable.

Introduction

Low-Density Lipoprotein Complexes

The human low-density lipoprotein (LDL) complex, which delivers cholesterol to cells by receptor-mediated endocytosis, consists of a core of about 1500 molecules of cholesteryl ester and triglyceride, surrounded by a 20 Å–thick shell of phospholipids, unesterified cholesterol and a single copy of apoprotein B 100 (~500,000 daltons).¹ Once internalized, LDL dissociates from its receptor and eventually appears in lysosomes.² Molecular Probes offers unlabeled LDL (L3486), which has been reported to be an effective vehicle for selectively delivering antitumor drugs to cancer cells,³ and an unlabeled LDL variant (L35354) that contains acetylated apoprotein (AcLDL). We also offer labeled LDL probes for studying the mechanisms of normal cholesterol delivery and internalization, as well as labeled AcLDL probes for the study of endothelial, microglial and other cell types that express receptors that specifically bind this modified LDL. See Table 1 for a full listing of Molecular Probes' labeled and unlabeled LDL and AcLDL products.

Fluorescent LDL Complexes

Molecular Probes offers LDL labeled with either DiI or the BODIPY FL fluorophore, highly fluorescent lipophilic dyes that diffuse into the hydrophobic portion of the LDL complex without affecting the LDL-specific binding of the apoprotein. The contrasting fluorescence of DiI LDL and fluorescein transferrin (T2871) permits their simultaneous use to follow the lysosomally directed pathway and the recycling pathways, respectively.⁴ As compared to DiI LDL, BODIPY FL LDL is more efficiently

excited by the 488 nm line of the argon-ion laser, making it better suited for flow cytometry and confocal laser scanning microscopy studies. Like our BODIPY FL C₅-ceramide (D3521), BODIPY FL LDL fluoresces somewhat in the red region, sometimes precluding its use for multicolor labeling with red fluorophores. Both the BODIPY FL LDL and DiI LDL have been used to investigate the binding specificity and partitioning of LDL throughout the *Schistosoma mansoni* parasite.⁵ Fluorescent LDL complexes have also proven useful in a variety of experimental systems to:

- Count the number of cell-surface LDL receptors, analyze their motion and clustering and follow their internalization⁶⁻⁸
- Demonstrate that fibroblasts grown continuously in the presence of DiI LDL (L3482) proliferate normally and exhibit normal morphology,⁹ making DiI LDL a valuable alternative to ¹²⁵I-labeled LDL for quantitating LDL-receptor activity¹⁰
- Identify LDL receptor-deficient Chinese hamster ovary (CHO) cell mutants¹¹
- Investigate the expression of LDL receptors in granulosa and luteal cells in primate and porcine ovarian follicles¹²⁻¹⁴
- Track the mobility of LDL receptors in an electric field¹⁵⁻¹⁷

Fluorescent Acetylated LDL Complexes

If the lysine residues of LDL's apoprotein have been acetylated, the LDL complex no longer binds to the LDL receptor,¹⁸ but rather is taken up by macrophage and endothelial cells that possess "scavenger" receptors specific for the modified LDL.^{19,20} Once the acetylated LDL (AcLDL) complexes accumulate within these cells, they assume an appearance similar to that of foam cells found in atherosclerotic plaques. It has now become routine to

identify endothelial cells and microglial cells in primary cell culture by their ability to take up fluorescent AcLDL.^{21,22} Molecular Probes' selection of dye-labeled AcLDL complexes is presented in Table 1.

Materials

Contents

Unlabeled LDL and AcLDL are supplied in units of 200 μ L 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. The labeled LDL and AcLDL complexes and conjugates are supplied in units of 200 μ L at a concentration of 1 mg/mL in the same buffer as the unlabeled LDL; the degree of labeling is indicated on the product label.

Molecular Probes prepares LDL and AcLDL products from fresh human plasma. If stored refrigerated and protected from light, our non-acetylated LDL products are stable for at least four to six weeks from the date of shipment. The AcLDL products, if stored properly, are stable for two to three months from the date of shipment. Multiple-unit discounts will be applied to standing orders of our LDL and AcLDL products. Because preparation of these complexes involves several variables, some batch-to-batch variability in the degree of labeling and fluorescence yield is to be expected.

Storage

Because these products are air-sensitive, they are packaged under argon. Upon arrival, store at 2–6°C, protected from light. **DO NOT FREEZE.**

Application

The binding of LDL to the LDL receptor requires the presence of either Ca²⁺ and Mn²⁺ and is inhibited by excess EDTA. The affinity of LDL for its receptor is lower at 37°C than at 4°C; however, the amount of LDL bound to receptors is two- to threefold higher at 37°C. To achieve half-maximal binding at 37°C, cells are typically incubated with approximately 10–15 μ g/mL LDL.⁸ Explicit labeling protocols for a variety of applications can be obtained from the literature.⁸

Precautions

These products contain human LDL. The venous blood from which the LDL is isolated has been tested for the presence of Hepatitis B Surface Antigen (HBsAg) and for HIV (HTLV III) antibody and was found to be negative for both. However, in accordance with good laboratory procedures, these products should be handled as if they are capable of transmitting hepatitis or other infectious agents.

References

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21. Eur J Cell Biol 60, 48 (1993);
22. J Cell Biol 99, 2034 (1984).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
L3486	low-density lipoprotein from human plasma (LDL) *2.5 mg/mL*	200 μ L
L35354	low-density lipoprotein from human plasma, acetylated (AcLDL) *2.5 mg/mL*	200 μ L
L23380	low-density lipoprotein from human plasma, acetylated, Alexa Fluor® 488 conjugate (Alexa Fluor® 488 AcLDL) *1 mg/mL*	200 μ L
L35353	low-density lipoprotein from human plasma, acetylated, Alexa Fluor® 594 conjugate (Alexa Fluor® 594 AcLDL) *1 mg/mL*	200 μ L
L3485	low-density lipoprotein from human plasma, acetylated, BODIPY® FL conjugate (BODIPY® FL AcLDL) *1 mg/mL*	200 μ L
L3484	low-density lipoprotein from human plasma, acetylated, Dil complex (Dil AcLDL) *1 mg/mL*	200 μ L
L3483	low-density lipoprotein from human plasma, BODIPY® FL complex (BODIPY® FL LDL) *1 mg/mL*	200 μ L
L3482	low-density lipoprotein from human plasma, Dil complex (Dil LDL) *1 mg/mL*	200 μ L

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