（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K205-M

Specification：96T(92 samples)

Measuring instrument：Microplate reader (530-570 nm)

Detection range：0.04-12 mmol/L

Elabscience® Low-Density Lipoprotein Cholesterol (LDL-C) Colorimetric Assay Kit (Double Reagents)

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service

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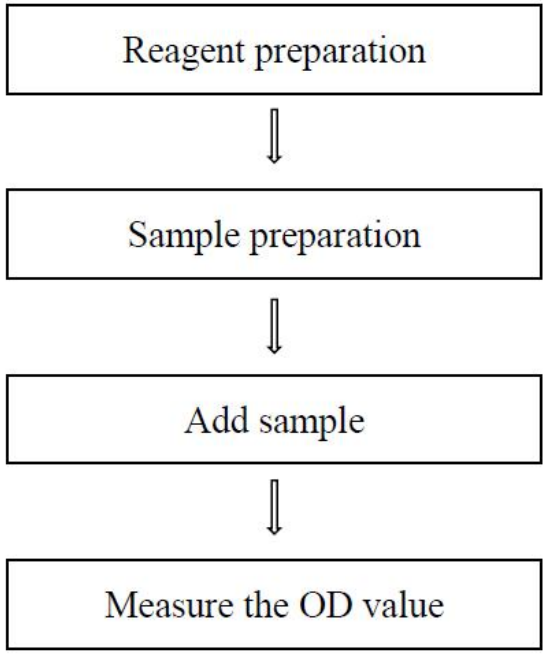
Assay summary

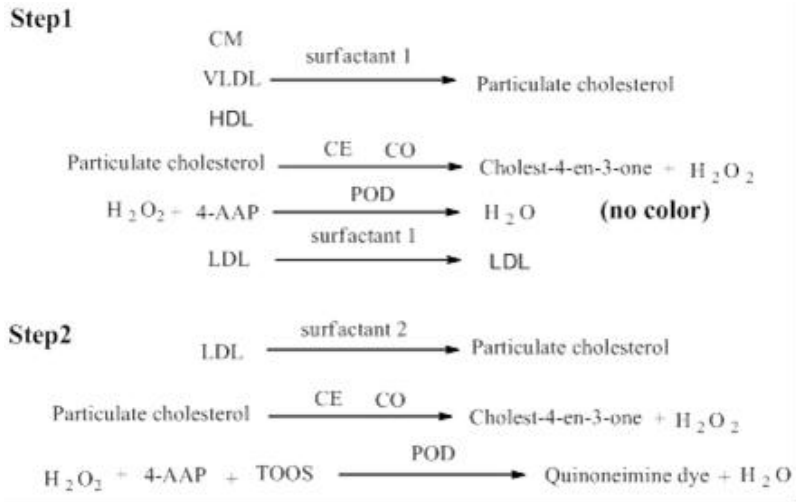


Intended use

This kit can be used to measure low-density lipoprotein cholesterol (LDL-C) content in serum, plasma, cells and tissue samples.

Detection principle

Lipoproteins (except LDL) such as HDL, CM, and VLDL change structure and dissociate under the action of surfactants. The released micronized cholesterol molecules react with cholesterol enzyme reagents, and the generated hydrogen peroxide is trapped in the absence of coupling agent. It is consumed without color development. At this time, the LDL particles are still intact, and then the reagent containing coupling agent is added, which can dissociate the LDL particles to release cholesterol, which is catalyzed by cholesterol esterase (CE) and cholesterol oxidase (CO) and produce hydrogen peroxide. Hydrogen peroxide is catalyzed by oxidase (POD) in the presence of 4-aminoantipyrine (4-AA) and phenol (T-OOS) to form a red quinone compound, because the cholesterol molecules of other lipoproteins have been removed, the shade is proportional to the amount of LDL-C.



Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Enzyme Working Solution 1	18 mL × 1 vial	2-8℃, 12 months shading light
Reagent 2	Enzyme Working Solution 2	6 mL × 1 vial	2-8℃, 12 months shading light
Reagent 3	Standard (Refer to the label for concentration)	Powder × 1 vial	2-8℃, 12 months shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (530 -570 nm, optimum wavelength: 546 nm)

Reagents:

Double distilled water, Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4), Isopropanol (AR)

Reagent preparation

① Equilibrate standard to 25℃ before use. Incubate enzyme working solution 1 and enzyme working solution 2 at 25℃ for 15 min with the amount required for the experiment, and the remaining reagents were stored at 2-8℃.

② The preparation of standard solution：
Dissolve one vial of standard with 200 μL of double distilled water. Store at 2-8°C for 2 weeks protected from light.

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL isopropanol with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

Cells:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 300-500 μL isopropanol with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect

supernatant and keep it on ice for detection.

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	1
Human plasma	1
Mouse serum	1
Rat plasma	1
Porcine serum	1
10% Mouse kidney tissue homogenate	1
10% Rat liver tissue homogenate	1
HepG2 cells	1

Note: The diluent of serum (plasma) is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). The diluent of animal tissue or cell is isopropanol. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① When adding the standards or samples, use the pipette to touch the bottom of microplate and add it.

② When measure OD value, there should be no bubbles in the well of microplate.

Operating steps

① Blank well: Add 5 μL of double distilled water to the corresponding wells.
Standard well: Add 5 μL of standard solution to the corresponding wells.
Sample well: Add 5 μL of sample to the corresponding wells.

② Add 180 μL of enzyme working solution 1 to the corresponding wells.

③ Mix fully, incubate at 37℃ for 5 min.

④ Measure the OD values of each well at 546 nm with microplate reader, as A_1.

⑤ Add 60 μL of enzyme working solution 2 to the corresponding wells.

⑥ Mix fully, incubate at 37℃ for 5 min.

⑦ Measure the OD values of each well at 546 nm with microplate reader, as A_2.

Calculation

The sample:

1. Serum (plasma) sample:

$$\text{LDL-C content (mmol/L)} = \frac{\Delta A_{sample}}{\Delta A_{standard}} \times c \times f$$

2. Tissue samples:

$$\text{LDL-C content (mmol/kg wet weight)} = \frac{\Delta A_{sample}}{\Delta A_{standard}} \times c \times f \div \frac{m}{V}$$

3. Cell samples:

$$\text{LDL-C content (μmol/10^6)} = \frac{\Delta A_{sample}}{\Delta A_{standard}} \times c \times f \div \frac{N}{V}$$

[Note]

ΔA_{sample}: ΔA of sample well –ΔA of blank well, ΔA： A_2- A_1

$\Delta A_{standard}$: ΔA of standard well –ΔA of blank well, ΔA： A_2- A_1

c: Concentration of standard.

f: Dilution factor of sample before test.

m: The weight of the sample, g.

V: Volume of isopropanol , mL.

N: The number of cells. For example, the number of cells is 5×10^6, N is 5.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	1.20	5.80	9.60
%CV	5.8	5.4	5.3

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	1.20	5.80	9.60
%CV	10.6	9.9	9.5

Sensitivity

The analytical sensitivity of the assay is 0.04 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Appendix II Example Analysis

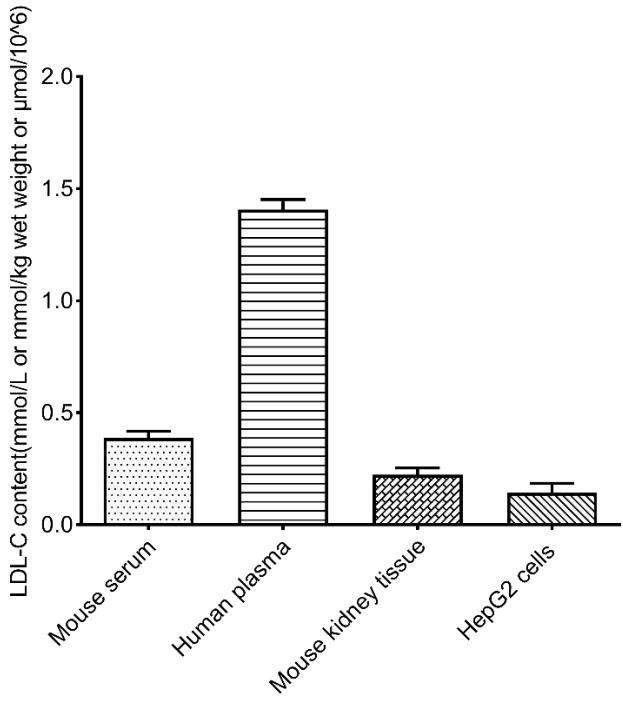
Example analysis：

Take 5 μL of mouse serum sample and carry the assay according to the operation steps. The results are as follows:

The average A_1 value of the blank is 0.043, the average A_2 value of the blank is 0.071, the average A_1 value of the standard is 0.061, the average A_2 value of the standard is 0.394, the average A_1 value of the sample is 0.052, the average A_2 value of the sample is 0.120, and the calculation result is:

$$\underset{(\text{mmol/L})}{\text{LDL-C}} = \frac{(0.120 - 0.052) - (0.071 - 0.043)}{(0.394 - 0.061) - (0.071 - 0.043)} \times 2.75\ \text{mmol/L} = 0.36\ \text{mmol/L}$$

Detect mouse serum, human plasma, 10% mouse kidney tissue homogenate and HepG2 cells, according to the protocol, the result is as follows：



Appendix III Publications

1. Zhicheng P , Jialan L , Liding Z ,et al.CircARCN1 aggravates atherosclerosis by regulating HuR-mediated USP31 mRNA in macrophages[J].Cardiovascular Research, 2024(13):13.DOI:10.1093/cvr/cvae148.
2. Lin-Yun Chen,Li-Wen Wang,Jie Wen,等.RNA-binding protein YBX3 promotes PPAR γ -SLC3A2 mediated BCAA metabolism fueling brown adipogenesis and thermogenesis[J].Molecular Metabolism, 2024, 90.DOI:10.1016/j.molmet.2024.102053.
3. Wang Y , Wang J , Zhou T ,et al.Investigating the potential mechanism and therapeutic effects of SLXG for cholesterol gallstone treatment[J].Phytomedicine, 2024, 132(000):16.DOI:10.1016/j.phymed.2024.155886.
4. Zou J , Song Q , Shaw P C ,et al.Tangerine Peel-Derived Exosome-Like Nanovesicles Alleviate Hepatic Steatosis Induced by Type 2 Diabetes: Evidenced by Regulating Lipid Metabolism and Intestinal Microflora[J].International Journal of Nanomedicine, 2024, 19.DOI:10.2147/IJN.S478589.
5. Yi M , Zhang Y , Zhang L ,et al.Screening of fish oil fatty acids for antihyperlipidemic activity based on network pharmacology and validation of synergistic efficacy in vitro[J].Food Bioscience, 2024, 58.DOI:10.1016/j.fbio.2024.103745.

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.