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

Catalog No：E-BC-K206-S

Specification：100Assays (Can detect 96 samples without duplication)

Measuring instrument：Spectrophotometer

Detection range：0.2-12 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
Tel: 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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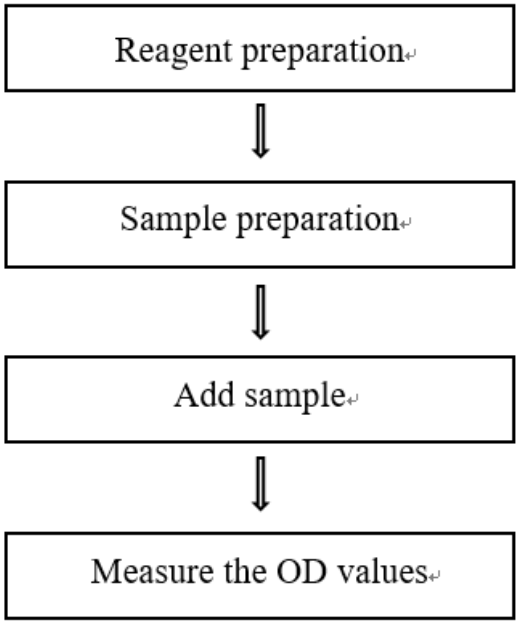
Assay summary



Intended use

This kit can be used for detection of low-density lipoprotein cholesterol (LDL-C) content in serum, plasma, cells, culture supernatant 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. The coloured substance have a maximum absorption peak at 546 nm. Measure the OD value at 546 nm and the LDL-C content in the sample can be calculated.

Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Enzyme Working Solution 1	75 mL ×1 vial	2-8℃, 12 months, shading light
Reagent 2	Enzyme Working Solution 2	25 mL ×1 vial	2-8℃, 12 months, shading light
Reagent 3	Cholesterol Standard (Refer to the label of reagent 3 for concentration)	Liquid ×1 vial	2-8℃, 12 months, shading light

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:

Spectrophotometer (600 nm), Micropipettor, Water bath, Incubator, Vortex mixer, Centrifuge

Reagents:

PBS (0.01 M, pH 7.4), absolute alcohol

Reagent preparation

Equilibrate all the reagents to room temperature before use.

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.

Culture supernatant sample: Collect the culture supernatant, centrifuge at 1000×g for 10 min, and take the supernatant for detection

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 PBS (0.01 M, pH 7.4) 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.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Note:

① If the tissue sample is not a high-fat sample, the homogenate medium should be normal saline or PBS.

② If the tissue sample is high-fat sample or partly high lipid sample, the homogenate medium should be absolute alcohol.

Cells:

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

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

③ Homogenize 10^6 cells in 300-500 μL PBS (0.01 M, pH 7.4) with a ultrasonic cell disruptor 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.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

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 is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① Protect the reagent from contamination of glucose, cholesterol, etc.

② The amount of reagent and sample can be increased and decreased proportionately according to the volume of cuvette

Operating steps

	Blank tube	Standard tube	Sample tube
Double distilled water (μL)	10		
Standard (μL)		10	
Sample (μL)			10
Enzyme Working Solution 1 (μL)	750	750	750
Mix fully and incubate at 37℃ for 5 min. Set the spectrophotometer to zero with distilled water and measure the absorbance value (A_1) of each tube at 546 nm wavelength with 0.5 cm optical path cuvette.			
Enzyme Working Solution 2 (μL)	250	250	250
Mix fully and incubate at 37℃ for 5 min. Set the spectrophotometer to zero with double distilled water and measure the absorbance value (A_2) of each tube at 546 nm wavelength with 0.5 cm optical path cuvette.			

Calculation

1. Serum (plasma) and other liquid sample:

$$\text{LDL-C content (mmol/L)} = \frac{(\text{Sample A2}-\text{SampleA1})-(\text{Blank A2}-\text{BlankA1})}{(\text{Standard A2}-\text{StandardA1})-(\text{BlankA2}-\text{BlankA1})} \times c \times f$$

2. Tissue and cell sample:

When homogenate medium is phosphate buffer or normal saline, the formula is as follows:

$$\text{LDL-C content (mmol/gprot)} = \frac{(\text{Sample A2-SampleA1})-(\text{Blank A2-BlankA1})}{(\text{Standard A2-StandardA1})-(\text{BlankA2-BlankA1})} \times c \times f \div C_{pr}$$

When homogenate medium is absolute alcohol, the formula is as follows:

$$\text{LDL-C content (mmol/g tissue)} = \frac{(\text{Sample A2-SampleA1})-(\text{Blank A2-BlankA1})}{(\text{Standard A2-StandardA1})-(\text{BlankA2-BlankA1})} \times c \times f \times V \div W$$

[Note]

C: Concentration of standard.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, gprot/L

V: The volume of absolute alcohol, L.

W: Weight of sample, g

Performance index

① The absorbance of the blank tube is ≤ 0.050 (Optical path is 0.5 cm).

② **Sensitivity:** The absorbance value △A is between 0.180~0.280 when testing 2.6 mmol/L samples.

③ **Linear range**: 0.2~12 mmol/L, r2 > 0.995

④ **Precision:** Intra CV ≤ 8%, Inter CV ≤ 10%.

⑤ **Stability:** This kit can be stored at 2~8℃ with shading light for 12 months and can be stable for 1 month since it is opened with the same storage condition.

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.