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

Catalog No：E-BC-K205-S

Specification：50 Assays(46 samples)/100 Assays(96 samples)

Measuring instrument：Spectrophotometer (546 nm)

Detection range：0.061-12 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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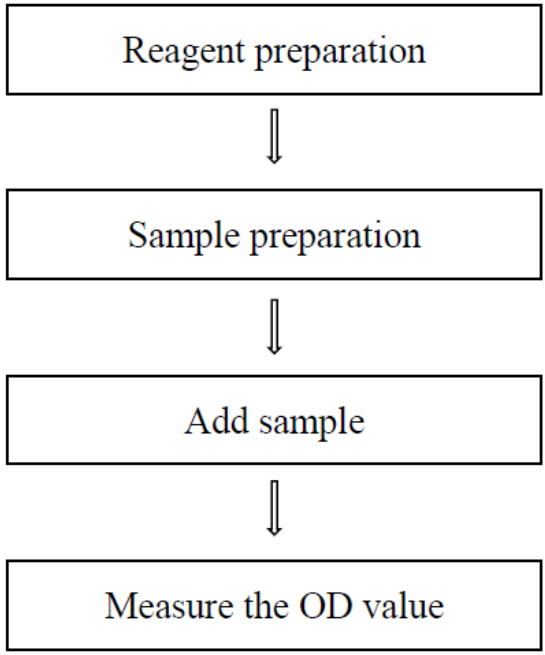
Assay summary



Intended use

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

Detection principle

Lipoproteins (except low-density lipoprotein cholesterol (LDL)) such as high density lipoprotein (HDL), chylomicron (CM), and very low density lipoprotein (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 shade of color is directly proportional to the LDL-C content.

Kit components & storage

Item	Component	Size 1 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Reaction Solution	45 mL ×1 vial	45 mL ×2 vials	2-8°C, 12 months shading light
Reagent 2	Working Solution	14 mL ×1 vial	28 mL ×1 vial	2-8°C, 12 months shading light
Reagent 3	Standard	Powder ×2 vials	Powder ×4 vials	2-8°C, 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 (546 nm), Vortex mixer, Incubator

Reagents:

Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4), Isopropyl alcohol (AR)

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of standard solution：

Dissolve one vial of standard with 200 μL of double distilled water, mix well to dissolve. Store at 2-8 ℃ 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 isopropyl alcohol with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min 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
Mouse plasma	1
Rat serum	1
Rat plasma	1
Porcine serum	1
Rabbit serum	1
Horse serum	1
10% Mouse lung tissue homogenization	1
10% Mouse kidney tissue homogenization	1
10% Mouse liver tissue homogenization	1

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

Operating steps

① Before detection, all reagents were incubated in a 25℃ incubator for 15 min in advance.

② Blank tube: Take 20 μL of double distilled water to the EP tubes.
Standard tube: Take 20 μL of standard solution to the EP tubes.
Sample tube: Take 20 μL of sample to the EP tubes.

③ Add 720 μL of reaction solution to each tube.

④ Mix fully by vortex mixer and incubate at 37℃ for 5 min protected from light. Set the spectrophotometer to zero with double distilled water and measure the absorbance at 546 nm with 1 cm optical path quartz cuvette, as A_1.

⑤ Add 240 μL of working solution to each tube.

⑥ Mix fully by vortex mixer and incubate at 37℃ for 5 min protected from light. Set the spectrophotometer to zero with double distilled water and measure the absorbance at 546 nm with 1 cm optical path quartz cuvette, as A_2.

Calculation

The sample:

1. Serum (plasma) sample:

$$\text{LDL-C content (mmol/L)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f$$

2. Tissue sample:

$$\text{LDL-C content (mmol/kg wet weight)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f \div \frac{m}{V}$$

[Note]

ΔA_1: $\triangle A_{sample} - \triangle A_{blank}$. $\Delta A = A_2 - A_1$.

ΔA_2: $\triangle A_{standard} - \triangle A_{blank}$. $\Delta A = A_2 - A_1$.

c: Concentration of standard. Its concentration is shown on the label, mmol/L.

f: Dilution factor of sample before tested.

m: The weight of the sample, g.

V: The volume of isopropyl alcohol in the preparation step of sample, mL.

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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)	1.00	1.20	2.00
%CV	1.1	4.8	1.6

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)	1.00	1.20	2.00
%CV	3.2	7.3	5.1

Sensitivity

The analytical sensitivity of the assay is 0.061 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

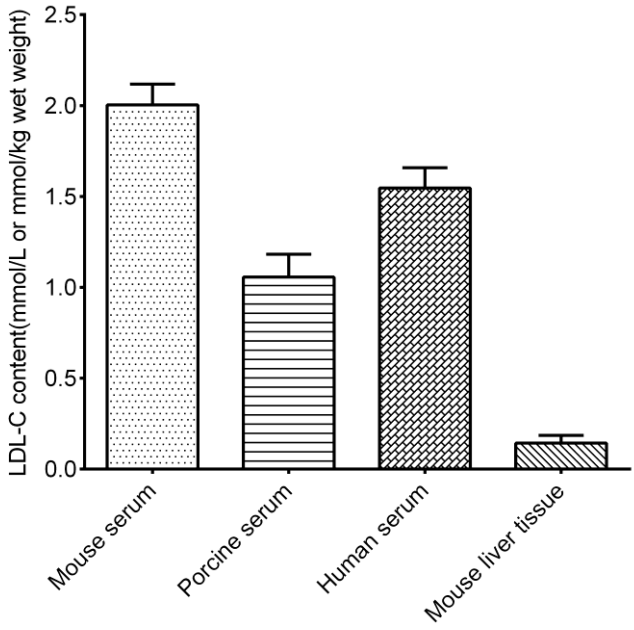
Example analysis：

Take 20 μL of human serum and carry the assay according to the operation steps. The results are as follows:

The A_1 of blank is 0.009, the A_2 of blank is 0.013, $\Delta A_{blank} = 0.013 - 0.009 = 0.004$. The A_1 of standard is 0.087, the A_2 of standard is 0.351, $\Delta A_{standard} = 0.351 - 0.087 = 0.264$. The A_1 of sample is 0.050, the A_2 of sample is 0.229, $\Delta A_{sample} = 0.229 - 0.050 = 0.179$, the concentration of standard is 2.3 mmol/L, and the calculation result is:

$$\text{LDL-C (mmol/L)} = (0.179 - 0.004) \div (0.264 - 0.004) \times 2.3 = 1.55 \text{ mmol/L}$$

Detect mouse serum, porcine serum, human serum and 10% mouse liver tissue homogenization, according to the protocol, the result is as follows：



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.