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

Catalog No：E-BC-K221

Specification：96T(92 samples)

Measuring instrument：Microplate reader, biochemical analyzer（546 nm）

Detection range：0.06-3.8 mmol/L

Elabscience® High-Density Lipoprotein Cholesterol (HDL-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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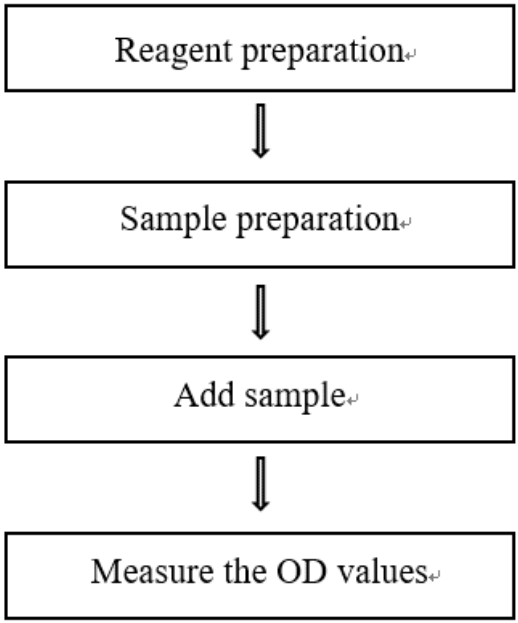
Assay summary

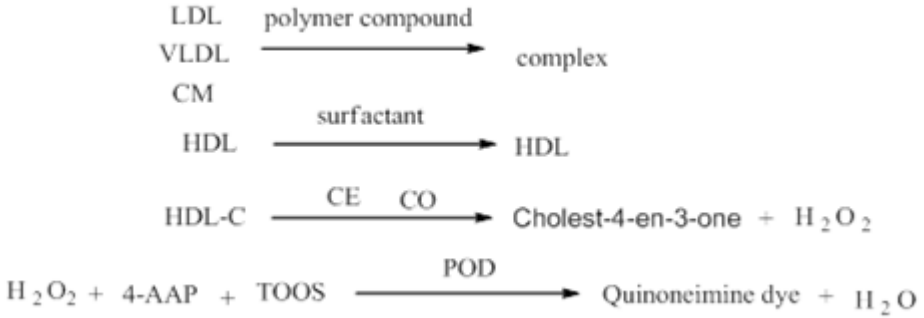


Intended use

This kit can be used to measure high-density lipoprotein cholesterol (HDL-C) content in serum, plasma samples.

Detection principle

CM, VLDL and LDL coagulate in a polyanionic environment to form a polymer and are masked by the polymer. High-density lipoprotein (HDL) form soluble compounds under the action of a surfactant, so that HDL-C can directly react with enzyme reagents containing cholesterol esterase (CE) and cholesterol oxidase (CO) to 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 HDL-C content in the sample can be calculated.



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 (546 nm) or Biochemical analyzer (546 nm), Micropipettor, Water bath, Incubator, Vortex mixer, Centrifuge

Reagents:

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

Reagent preparation

① Equilibrate standard to room temperature 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 ℃ 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.

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

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 Prevent the formulation of bubbles when adding the liquid to the microplate.

② Protect the reagent from contamination of glucose, cholesterol, etc.

③ The amount of reagent and sample can be increased and decreased proportionately according to the volume of cuvette.

Operating steps

1. Operate with 96 wells microplate reader.

	Blank well	Standard well	Sample well
Double distilled water (μL)	5		
Standard (μL)		5	
Sample (μL)			5
Enzyme working solution 1 (μL)	180	180	180
Mix fully and incubate at 37℃ for 5 min. Measure the OD value (A_1) at 546 nm with microplate reader.			
Enzyme working solution 2 (μL)	60	60	60
Mix fully and incubate at 37℃ for 5 min. Measure the OD value (A_2) at 546 nm with microplate reader. $\Delta A=A_2-A_1$.			

2. Operate with Automatic biochemical analyzer

a. Setting parameter

Main wavelength	546 nm
Reaction type	Terminal method
Reaction direction	Up reaction (+)

b. Operation steps

Sample/ Double distilled water (μL)	5
Enzyme working solution 1 (μL)	180
Mix fully and incubate at 37℃ for 5 min. Measure the OD value (A_1) at 546 nm with biochemical analyzer.	
Enzyme working solution 2 (μL)	60
Mix fully and incubate at 37℃ for 5 min. Measure the OD value (A_2) at 546 nm with biochemical analyzer. $\Delta A=A_2-A_1$.	

Calculation

The sample:

Serum (plasma) and other liquid sample:

Operated with microplate reader:

$$\text{HDL-C (mmol/L)} = \frac{\Delta A_{sample} - \Delta A_{blank}}{\Delta A_{Standard} - \Delta A_{blank}} \times c \times f$$

Operated with automatic biochemical analyzer:

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

[Note]

c: Concentration of standard.

f: Dilution factor of sample before test.

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)	0.54	1.60	2.70
%CV	3.3	3.0	2.7

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)	0.54	1.60	2.70
%CV	4.7	5.6	4.7

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 95%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mmol/L)	0.85	2.4	3.3
Observed Conc. (mmol/L)）	0.8	2.2	3.1
Recovery rate (%)	99	92	94

Sensitivity

The analytical sensitivity of the assay is 0.06 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

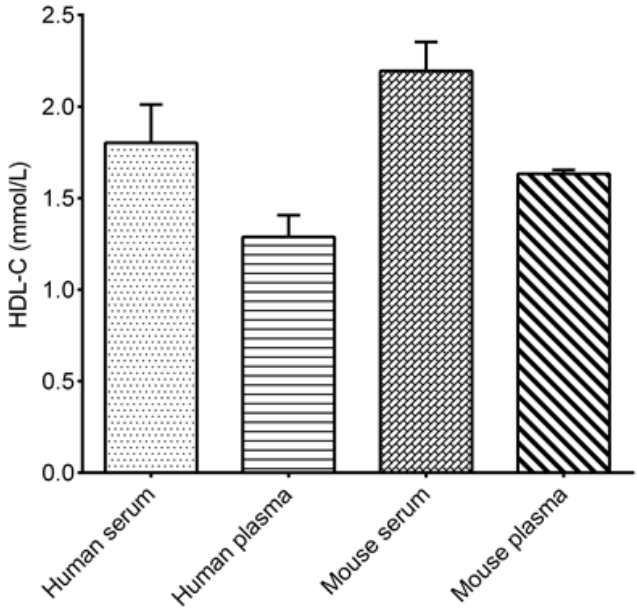
Example analysis:

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

The average OD value of the blank (A_1) is 0.043, the average OD value of the blank (A_2) is 0.059, the average OD value of the standard (A_1) is 0.064, the average OD value of the standard (A_2) is 0.172, the average OD value of the sample (A_1) is 0.050, the average OD value of the sample (A_2) is 0.246, and the calculation result is:

$$\text{HDL-C (mmol/L)} = \frac{(0.246 - 0.050) - (0.059 - 0.043)}{(0.172 - 0.064) - (0.059 - 0.043)} \times 1.1 \text{ mmol/L} = 2.15 \text{ mmol/L}$$

Detect human serum, human plasma, mouse serum and mouse plasma 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.