

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

Catalog No: E-BC-K222-S

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

Measuring instrument: Spectrophotometer

Detection range: 0.065-3.8 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

Tel: 1-832-243-6086

Fax: 1-832-243-6017

Email: techsupport@elabscience.com

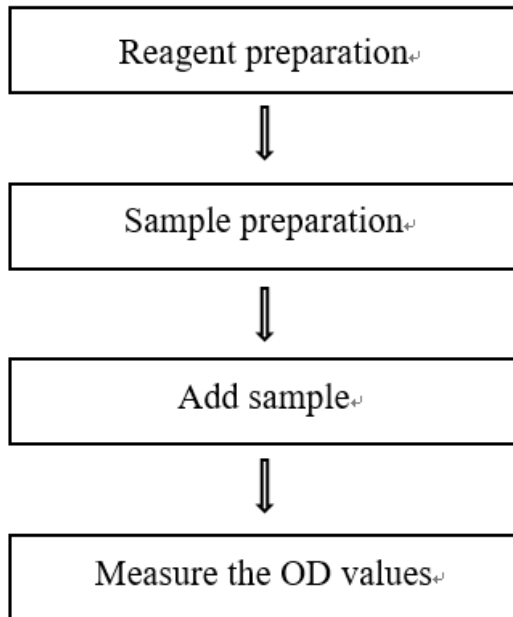
Website: www.elabscience.com

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

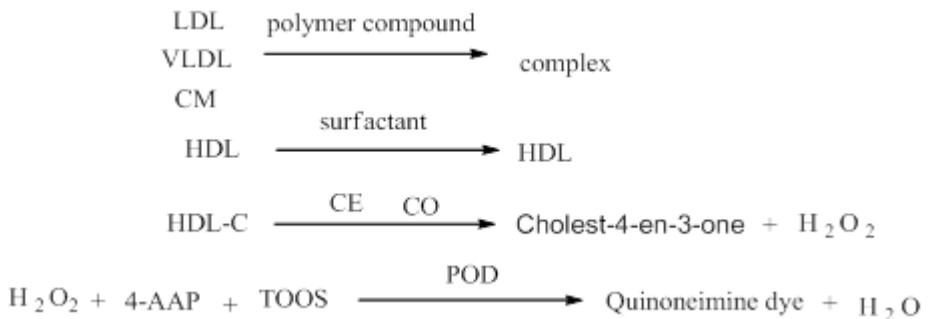


Intended use

This kit can be used for detection of high-density lipoprotein cholesterol (HDL-C) content in serum, plasma, cells, culture supernatant and tissue 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 600 nm. Measure the OD value at 600 nm and the HDL-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°C, 12 months, shading light
Reagent 2	Enzyme Working Solution 2	25 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 3	Cholesterol Standard (Refer to the label of reagent 3 for concentration)	Powder × 1 vial	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 (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°C for a month.

Culture supernatant sample: Collect the culture supernatant, centrifuge at $1000\times g$ 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°C .
- ④ Centrifuge at $10000\times g$ for 10 min at 4°C 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°C .
- ④ Centrifuge at $10000\times g$ for 10 min at 4°C 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
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 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 well	Standard well	Sample well
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°C for 5 min. Set the spectrophotometer to zero with double distilled water and measure the OD value (A_1) of each tube at 600 nm with 0.5 cm optical path cuvette.			
Enzyme Working Solution 2 (μL)	250	250	250
Mix fully and incubate at 37°C for 5 min. Set the spectrophotometer to zero with double distilled water and measure the OD value (A_2) of each tube at 600 nm with 0.5 cm optical path cuvette.			

Calculation

1. Serum (plasma) and other liquid sample:

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

2. Tissue and cell sample:

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

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

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

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

[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 blank tube is ≤ 0.010 (optical path = 0.5 cm).
- ② **Linear range:** 0.065~3.8 mmol/L, $r^2 > 0.995$.
- ③ **Sensitivity:** The absorbance value of ΔA is between 0.087~0.153 when testing 1.3 mmol/L samples
- ④ **Accuracy:** Relative deviation $\leq 10\%$.
- ⑤ **Precision:** Intra-CV $\leq 3\%$, Inter-CV $\leq 5\%$
- ⑥ **Stability:** The validity of kit is 12 months when stored at 2-8°C in the dark. It is stable for 1 months when stored at 2-8°C in the dark after opening.

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.

