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

Catalog No：E-BC-F035

Specification：48T(32 samples)96T(80 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：0.15-295 μmol/L

Elabscience® High-Density Lipoprotein Chlesterol (HDL-C) Fluorometric Assay Kit

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.

Table of contents

Assay summary 3

Intended use 4

Detection principle 4

Kit components & storage 5

Materials prepared by users 5

Reagent preparation 6

Sample preparation 7

Operating steps 9

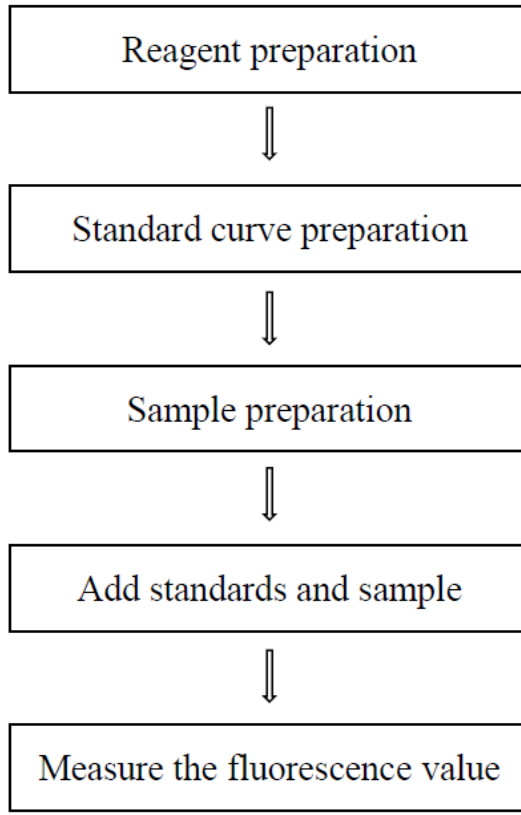
Calculation 10

Appendix I Performance Characteristics 11

Appendix II Example Analysis 13

Statement 14

Assay summary



Intended use

This kit can be used to measure high-density lipoprotein chlesterol (HDL-C) content in serum (plasma), animal tissue and cell samples.

Detection principle

High-density lipoprotein cholesterol (HDL-C), mainly synthesized in the liver, is an anti-atherosclerotic lipoprotein composed of apolipoprotein, phospholipid, cholesterol and fatty acids. The role of HDL-C is to transport phospholipids and cholesterol, which can transport cholesterol from extrahepatic tissues to the liver for metabolism, and be excreted from the body by bile acids. Plasma level of HDL-C are inversely associated with the risk of cardiovascular disease. HDL can take cholesterol from cell membranes, catalyze it into cholesterol esters by lecithin cholesterol acyltransferase, and then transfer the cholesterol ester to very low density lipoproteins and low density lipoproteins. The cholesterol content of high-density lipoprotein is relatively fixed, about 20-30% of the total cholesterol of the human body.

After a series of reactions of HDL-C, the product can form a complex with the fluorescent probe. The fluorescence value of the sample at the excitation wavelength of 535 nm and the emission wavelength of 587 nm can be measured, and the content of HDL-C in the sample can be calculated.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Reaction Solution	10 mL ×1 vial	20 mL ×1 vial	2-8℃, 12 months shading light
Reagent 2	Enzyme Solution	5 mL ×1 vial	10 mL ×1 vial	2-8℃, 12 months shading light
Reagent 3	Chromogenic Agent	0.2 mL ×1 vial	0.4 mL ×1 vial	2-8℃, 12 months shading light
Reagent 4	Standard	0.2 mL ×1 vial	0.4 mL ×1 vial	2-8℃, 12 months
	Black 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:

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Incubator (37°C)

Reagents:

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

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of working solution:

Before testing, please prepare sufficient working solution according to the test wells. For example, prepare 130 μL of working solution (mix well 125 μL of enzyme solution and 5 μL of chromogenic agent). Store at 2-8 ℃ protected from light and used up within one day.

③ The preparation of 295 μmol/L standard solution:

Before testing, please prepare sufficient 295 μmol/L standard solution. For example, prepare 1000 μL of 295 μmol/L standard solution (mix well 50 μL of standard and 950 μL of double distilled water). Store at 2-8 ℃ for a week.

④ The preparation of standard curve:

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 295 μmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 59, 118, 147.5, 177, 206.5, 236, 295 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**59**	**118**	**147.5**	**177**	**206.5**	**236**	**295**
295 μmol/L Standard (μL)	0	40	80	100	120	140	160	200
Double distilled water (μL)	200	160	120	100	80	60	40	0

Sample preparation

① Sample preparation

Serum or plasma samples: detect directly.

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 (AR) 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.

Cell (adherent or suspension) samples:

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

② Take 1×10^6 cells with 200 μL isopropyl alcohol (AR), ultrasound was performed for 10 min.

③ Centrifuge at 10000×g for 10 min at 4℃ 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	8-15
Rat serum	2-6
Porcine serum	4-10
Mouse serum	4-10
Rabbit serum	4-10
Horse serum	2-6
10% Mouse liver tissue homogenate	4-8
10% Mouse lung tissue homogenate	6-12
1×10^6 Molt-4 cells	1
1×10^6 Jurkat cells	1
1×10^6 3T3 cells	1
1×10^6 Hela cells	1
1×10^6 HL-60 cells	1
1×10^6 293T cells	1

Note: The diluent for serum (plasma) is normal saline (0.9% NaCl). The diluent for tissue and cell samples are isopropyl alcohol (AR). For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Before the test, incabate the reaction solution and working solution at 37℃ for 15min.

② Standard well: add 10 μL of standard with different concentrations into the well.

Sample well: add 10 μL of sample into the well.

③ Add 180 μL of reaction solution into each well.

④ Mix fully with fluorescence microplate for 3s. Incubate at 37 ℃ for 5 min protected from light. Add 60 μL of working solution into each well.

⑤ Mix fully with fluorescence microplate for 3s. Incubate at 37 ℃ for 5 min protected from light. Measure the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean fluorescence value of the blank (Standard #①) from all standard readings. This is the absoluted fluorescence value.

3. Plot the standard curve by using absoluted fluorescence value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum (plasma) samples:

$$\text{HDL-C content (μmol/L)} = \frac{\Delta F - b}{a} \times f$$

2. Tissue sample:

$$\text{HDL-C content (μmol/kg wet weight)} = \frac{\Delta F - b}{a} \div \frac{m}{V} \times f$$

3. Cell sample:

$$\text{HDL-C content (nmol/10^6)} = \frac{\Delta F - b}{a} \div \frac{n}{V} \times f$$

[Note]

ΔF: $\Delta F = F_{sample} - F_{blank}$. ($F_{blank}$ is the fluorescence value when the standard concentration is 0).

m: The weight of tissue, g.

V: The volume of normal saline in the preparation of sample, mL.

n: The number of cell sample/10^6.

f: Dilution factor of sample before tested.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	50.00	80.00	110.00
%CV	2.1	2.3	2.4

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	50.00	80.00	110.00
%CV	7.1	7.6	8.7

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 96.3%.

	Standard 1	Standard 2	Standard 3
Expected Conc(μmol/L)	50	80	110
Observed Conc(μmol/L)	49.5	76.0	104.5
Recovery rate (%)	99	95	95

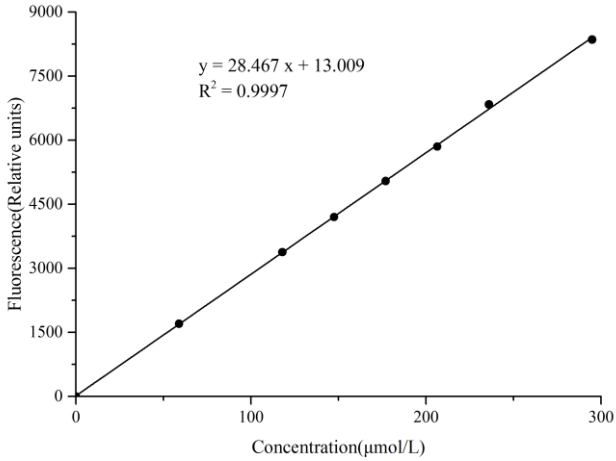
Sensitivity

The analytical sensitivity of the assay is 0.15 μmol/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.

2. Standard curve:

As the fluorescence value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (μmol/L)	0	59	118	147.5	177	206.5	236	295
Fluorescence value	335	2027	3745	4422	5357	6142	7109	8680
	354	2066	3706	4669	5422	6249	7258	8727
Average fluorescence value	345	2046	3725	4546	5390	6195	7183	8703
Absoluted fluorescence value	0	1701	3380	4201	5045	5850	6838	8358



Appendix II Example Analysis

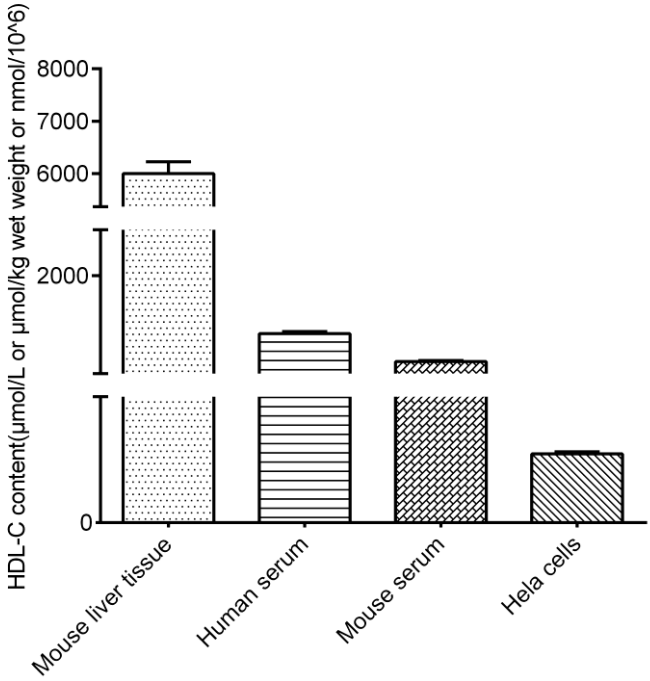
Example analysis:

Take 10 μL of 10% mouse liver tissue homogenate supernatant which dilute for 4 times and carry the assay according to the operation steps. The results are as follows: Standard curve: y = 28.467 x + 13.009, the average fluorescence value of the sample well is 4872, the average fluorescence value of the blank well is 345, and the calculation result is:

HDL-C content (μmol/kg wet weight) = (4872 - 345 - 13.009) ÷28.467 ×0.9 ÷0.1 ×4

= 5708.49 μmol/kg wet weight

Detect 10% mouse liver tissue homogenate (dilute for 4 times), human serum (dilute for 5 times), mouse serum (dilute for 5 times) and 1×10^6 Hela cells 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.