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

Catalog No：E-BC-K004-M

Specification：48T(44 samples)/96T(92 samples)/96T×5(476 samples)

Measuring instrument：Microplate reader（500-520 nm）

Detection range：0.07-24 mmol/L

Elabscience® Free Cholesterol (FC)

Colorimetric 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
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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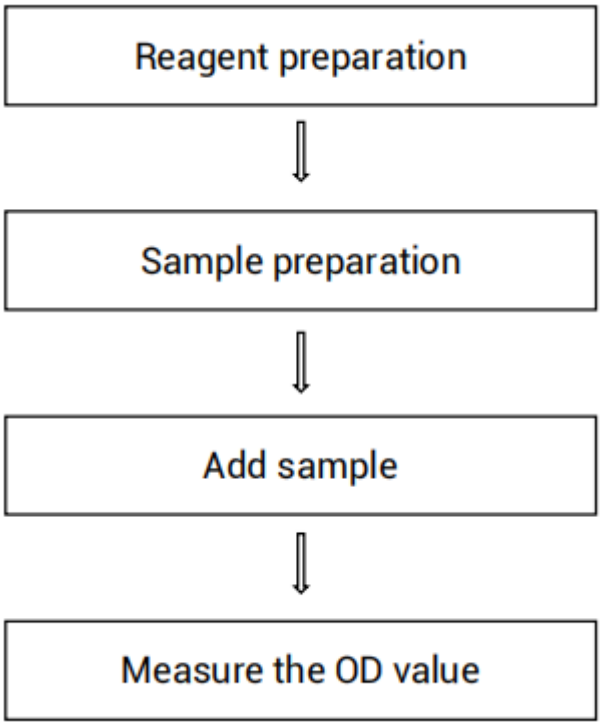
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Assay summary

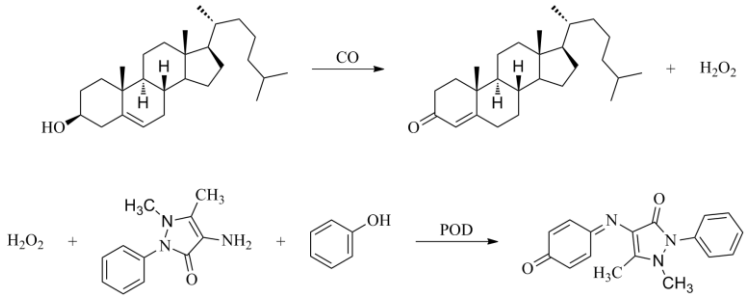


Intended use

This kit applies the COD-PAP method and it can be used for in vitro determination of free cholesterol content in serum, plasma, tissue samples.

Detection principle

Free cholesterol produces 4-cholestenone and hydrogen peroxide under the oxidation of cholesterol oxidase. In the presence of 4-aminoamylpyridine and phenol, peroxidase catalyze hydrogen peroxide to form red quinone compounds of benzoquinone imine phenizone. The color depth of the generated quinones is directly proportional to the cholesterol content.



Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (96 T×5)	Storage
Reagent 1	Enzyme Working Solution	15 mL×1vial	30 mL×1 vial	50 mL×3 vials	2-8°C, 12 months, shading light
Reagent 2	5.17 mM Cholesterol Standard	0.2 mL×1vial	0.2 mL×1 vial	0.5 mL×1 vial	2-8°C, 12 months
	Microplate	48 wells×1	96 wells×1	96 wells×5	No requirement
	Plate Sealer	2 pieces		7 pieces	
	Sample Layout Sheet	1 piece		7 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 (500-520 nm, optimum wavelength: 510 nm),
Micropipettor, Vortex mixer, Incubator, Centrifuge

Reagents:

Double distilled water, Normal saline (0.85% NaCl), PBS (0.01 M, pH 7.4), Anhydrous ethanol

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.

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 anhydrous ethanol with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min 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 plasma	1
Human serum	1
Rat serum	1
Mouse serum	1
Rabbit serum	1
10% Rat kidney tissue homogenate	1

Note: The diluent is normal saline (0.85% 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 the reagents is added into the microplate.
② Equilibrate all the reagents to room temperature before use.

Operating steps

① Blank well: add 5 μL of double distilled water into the well.
Standard well: add 5 μL of standard solution into the well.
Sample well: add 5 μL of sample into the well
② Add 250 μL of enzyme working solution to each well.
③ Mix thoroughly, incubate at 37°C for 10 min, measure absorbance value at 510 nm with microplate reader.

Calculation

The sample:

1. Serum (plasma) sample and other liquid samples:

$$\text{Free cholesterol content (mmol/L)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f$$

2. Tissue sample:

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

[Note]

ΔA_1: OD_{sample}-OD_{blank}

ΔA_2: $OD_{standard}$-OD_{blank}

c: the concentration of standard, 5.17 mmol/L.

f: Dilution factor of sample before tested.

m: the weight of tissue sample, g.

V: the volume of anhydrous ethanol, mL.

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	10.00	17.00
%CV	2.5	1.9	1.3

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	10.00	17.00
%CV	5.7	4.3	5.6

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 100%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mmol/L)	8	18	20
Observed Conc. (mmol/L)	7.9	17.6	18.8
Recovery rate (%)	99	98	94

Sensitivity

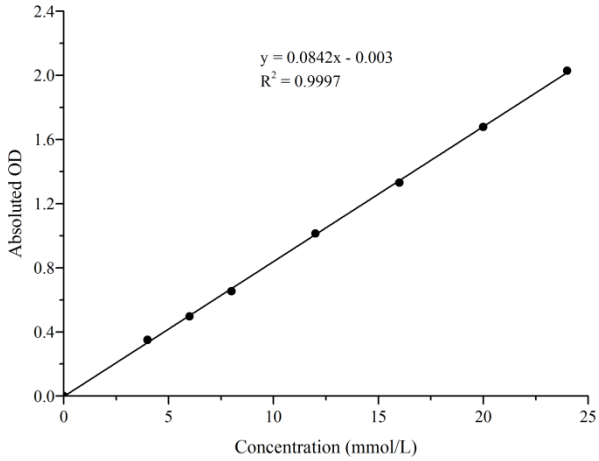
The analytical sensitivity of the assay is 0.07 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

(It doesn't need to prepare the standard curve for this kit and the provided standard curve is for reference only)

As the OD 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 (mmol/L)	0	4	6	8	12	16	20	24
OD value	0.078	0.420	0.567	0.734	1.082	1.380	1.767	2.122
	0.077	0.436	0.581	0.728	1.102	1.436	1.746	2.092
Average OD	0.078	0.428	0.574	0.731	1.092	1.408	1.756	2.107
Absoluted OD	0.000	0.351	0.497	0.653	1.014	1.331	1.679	2.029



Appendix Π Example Analysis

Example analysis：

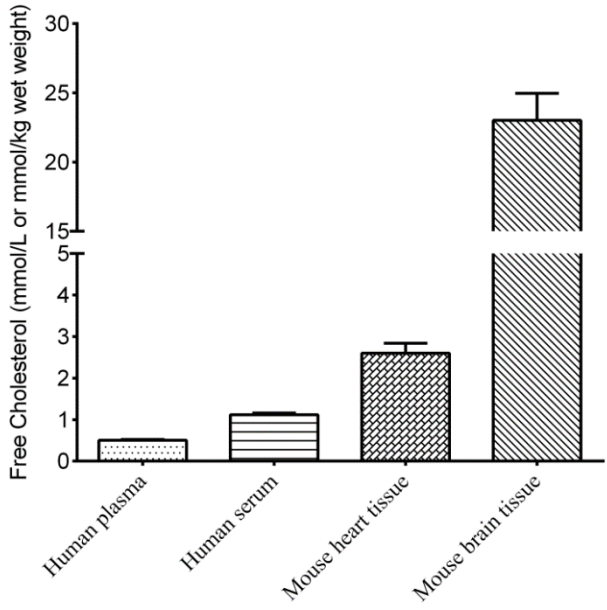
For mouse brain tissue, take 5 μL of 10% mouse brain tissue homogenate and carry the assay according to the operation steps. The results are as follows:

the average OD value of the sample is 0.318, the average OD value of the blank is 0.089, the average OD value of the standard is 0.519, and the calculation result is:

$$\text{Free cholesterol content (mmol/kg wet weight)} = (0.318\text{-}0.089) \div (0.318 - 0.089) \times 5.17 \times 0.9 \div 0.1$$

$$= 24.78 \text{ mmol/ kg wet weight}$$

Detect human plasma, human serum, 10% mouse heart tissue homogenate and 10% mouse brain tissue homogenate according to the protocol, the result is as follows：



Appendix III Publications

1. Zhicheng P , Jialan L , Liding Z ,et al.CircARCN1 aggravates atherosclerosis by regulating HuR-mediated USP31 mRNA in macrophages[J].Cardiovascular Research, 2024(13):13.DOI:10.1093/cvr/cvae148.
2. Baldea I , Moldovan R , Nagy A L ,et al.Ketoconazole-Fumaric Acid Pharmaceutical Cocrystal: From Formulation Design for Bioavailability Improvement to Biocompatibility Testing and Antifungal Efficacy Evaluation[J].International Journal of Molecular Sciences, 2024, 25(24).DOI:10.3390/ijms252413346.

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.