Total Cholesterol (TC) Colorimetric Assay Kit
(Single Reagent, COD-PAP Method)

Catalog No: E-BC-K109-S

Method: Colorimetric method

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

Instrument: Spectrophotometer

Sensitivity: 0.09 mmol/L

Detection range: 0.09-25.85 mmol/L

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

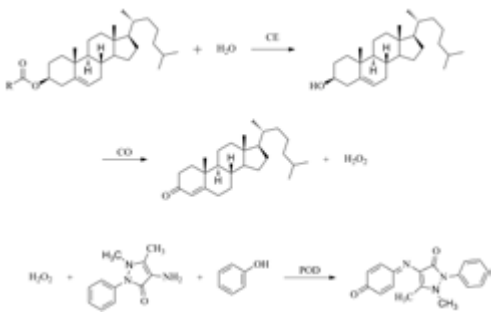
This kit applies the COD-PAP method and it can be used for in vitro determination of total cholesterol (TC) content in serum, plasma, animal tissue, cells and other samples.

▲Background

Cholesterol is a kind of sterol and lipid in cell membrane. Most of cholesterol in blood exists in the form of cholesterol ester. Human plasma lecithin-cholesterol acyltransferase is an enzyme that catalyzes the formation of cholesterol ester. Cholesterol synthesized or deposited in peripheral cells returns to the liver through the reverse cholesterol transport system for reuse or re-conversion into bile acids.

▲Detection principle

Total cholesterol includes free cholesterol and cholesterol esters. Cholesterol ester can be hydrolyzed by cholesterol esterase to produce cholesterol and free fatty acid. Cholesterol is oxidized by cholesterol oxidase to produce Δ4-cholestenone and hydrogen peroxide. In the presence of 4-aminoamylpyridine and phenol, hydrogen peroxide catalyze peroxidase to form red quinone compounds of benzoquinone imine phenizone.The color depth of the generated quinone is directly proportional to the cholesterol content. The absorbance values of the standard tube and the sample tube are measured respectively, and the cholesterol content in the sample can be calculated.


CE
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▲Kit components & storage

Item	Component	Concentration	Specification	Storage
Enzyme Working Solution	Good's Buffer	50 mmol/L, pH7.0	60 mL × 2 vials	2-8°C , 6 months, shading light
	Phenol	5 mmol/L		
	4-AAP	0.3 mmol/L		
	Cholesterol esterase	≥ 200 KU/L		
	Cholesterol oxidase	≥ 100 KU/L		
	Peroxidase	≥ 3 KU/L		
5.17 mM Cholesterol Standard			0.5 mL × 1 vial	2-8°C , 6 months

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.
If you have any problem, please contact our Technical Service Center for help.
Phone: 240-252-7368(USA) Fax: 240-252-7376(USA)
Email: techsupport@elabscience.com
Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

▲Materials prepared by users

Instruments

Spectrophotometer (510 nm), Micropipettor, Incubator, Centrifuge

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL)

Reagents

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

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲The key points of the assay

1. Protect the reagent from contamination of glucose, cholesterol, etc.
2. Since the volume of standard and sample is 10 μL, it is necessary to adhere to the wall of the EP tubes when adding the liquid to reduce the error.
3. When measuring low content samples such as cells, the volume of sample should be increased to 20 μL, and the volume of blank well and standard well should be increased at the same time.

Pre-assay preparation

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

Sample requirements

The samples should not contain reducing substances such as ascorbic acid or glutathione, otherwise the generated hydrogen peroxide will be consumed, and competitive inhibition will result in low measurement results.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.09-25.85 mmol/L).

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	1
Mouse serum	1
Rat plasma	1
10% Mouse liver tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse heart tissue homogenate	1
HepG2 cells	1

Note: The diluent of serum (plasma) is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4); The diluent of animal tissue and cells is isopropanol.

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	510 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Operating steps

1. Blank tube: Add 10 μL of double distilled water into a 2 mL EP tube.
   Standard tube: Add 10 μL of reagent 2 into a 2 mL EP tube.
   Sample tube: Add 10 μL of sample into a 2 mL EP tube.
2. Add 1000 μL of reagent 1 and mix fully.
3. Incubate at 37°C for 10 min. Set the spectrophotometerto zero with double distilled water and measure the OD values of each tube at 550 nm with 0.5 cm diameter cuvette.

▲Operation table

	Blank tube	Standard tube	Sample tube
Double distilled water (μL)	10		
Standard (μL)		10	
Sample (μL)			10
Reagent 1 (μL)	1000	1000	1000
Mix thoroughly and incubate at 37°C for 10 min. Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 510 nm with 0.5 cm optical path quartz cuvette.			

▲Calculation

1. Serum (plasma) and other liquid sample:

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

2. Tissue sample:

$$\text{TC content(mmol/g fresh weight)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f \div \frac{m}{V}$$

3. Cells sample:

$$\text{TC content}(\mu\text{mol}/10^6) = \frac{\Delta A_1}{\Delta A_2} \times c \times f \div \frac{N}{V}$$

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, mg.

V: the volume of isopropanol, mL.

N: the number of cells. For example, the number of cells is $5*10^6$, N is 5.

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	0.09-25.85 mmol/L	Average intra-assay CV (%)	1.1
Sensitivity	0.09 mmol/L	Average inter-assay CV (%)	2.8
Average recovery rate (%)	102		

▲Example analysis

Take 10 μL of human serum, carry the assay according to the operation table.

The results are as follows:

The average OD value of the sample is 0.186, the average OD value of the blank is 0.028, the average OD value of the standard is 0.210, and the calculation result is:

$$\text{TC content(mmol/L)} = \frac{0.186-0.028}{0.210-0.028} \times 5.17 = 4.49 \text{ mmol/L}$$

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80℃ for a month.

▲Plasma

Take fresh blood into the tube which has anticoagulant, centrifuge at 700-1000 g for 10 min at 4℃ . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80℃ for a month.

▲Hydrothorax

Take fresh hydrothorax into the tube which has anticoagulant. centrifuge at 10000 g for 10 min at 4℃ . Take the supernatant to preserve it on ice for detection. If not detected on the same day, the sample can be stored at -80℃ for a month.

▲Tissue sample

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8℃ . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4℃ . Take the supernatant to preserve it on ice for detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for a month.

▲Cells

Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (2×10^6): homogenization medium (μL) =1: 200. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80℃ for a month.

Note:

1. Homogenized medium: Isopropanol.

2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.
Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

(3) Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2 s/time, interval for 3 s, the total time is 5 min).

▲Note for sample

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
3. If a lysis buffer is used to prepare tissue or cell homogenates, there is a possibility of causing a deviation due to the introduced chemical substance.