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

Total Cholesterol (TC) Assay Kit (COD-PAP Method)

Catalog No.: BC00054

Size: 100T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

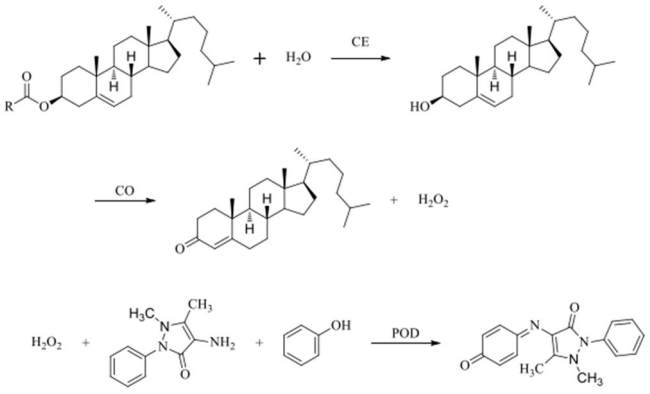
Product Name	Total Cholesterol (TC) Assay Kit (COD-PAP Method)
Detection Method	Colorimetric
Sample Type	Tissue, serum, plasma and other samples
Assay Type	Quantitative
Detection Instrument	Microplate reader (510 nm)

Product Introduction

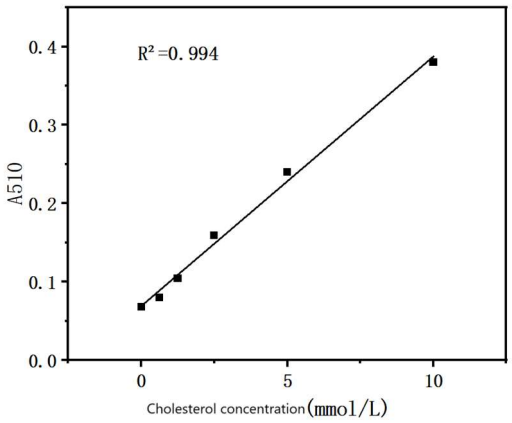
Total cholesterol refers to the sum of cholesterol contained in all lipoproteins in the blood. The total cholesterol level of a population mainly depends on genetic factors and lifestyle. Total cholesterol includes free cholesterol and cholesterol esters, and the liver is the main organ for synthesis and storage.

Principle

Total cholesterol (TC) includes free cholesterol and cholesterol esters. Cholesterol esters can be hydrolyzed into cholesterol and free fatty acids by cholesterol esterase (CE). Cholesterol generates △4-cholestenone and hydrogen peroxide under the oxidation of cholesterol oxidase (CO). In the presence of 4-aminoantipyrine and phenol, hydrogen peroxide is catalyzed by peroxidase (POD) to generate red quinone compounds of benzoquinone imine phenazone. The color depth is proportional to the TC content. There is a special absorption peak at 510nm. Based on this, the total cholesterol content in the sample can be calculated from the absorbance value. The detection principle is shown in the figure below:



The following standard curve is for reference only:



Components

No.	Components	Size (100T)	Storage
Reagent 1	Enzyme Working Solution	30 mL	-20℃, store at 2-8°C after opening.
Reagent 2	10 mmol/L Cholesterol Standard Solution	0.2 mL	-20℃, store at 2-8°C after opening.
Consumable 1	Microplate	1 plate	RT
Consumable 2	Plate Sealer	2 pieces	RT

Storage

The unopened kit can be stored at -20℃ for 12 months, and after opening, it can be stored at 2-8℃ for 6 months.

Preparation

- **Sample handling**

1. Liquid samples such as serum and plasma: Can be measured directly.
2. Tissue samples: Routine homogenization (**homogenization medium is anhydrous ethanol**).
3. Sample dilution: Before the formal test, 2-3 samples with large expected differences

can be selected and diluted into different concentrations for preliminary experiments. According to the results of the preliminary experiment, the dilution factor is selected in combination with the linear range of this kit: 0-10 mmol/L.

Note: The diluent for serum (plasma) is physiological saline (0.9% NaCl) or PBS (0.01 M, pH 7.4); the diluent for tissue samples is anhydrous ethanol. **Reducing substances such as ascorbic acid and glutathione cannot be added to the samples.**

- **Preparation of the kit**

1. Take out all reagents and return to room temperature before use.
2. Dilution of different concentrations of standards: Dilute the standard solution to different concentrations such as 10, 5, 2.5, 1.25, 0.625, and 0 (blank well) mmol/L using anhydrous ethanol according to the half-dilution method.

Operation process

1. Blank wells: Take 2.5 μL of anhydrous ethanol and add to the A wells of the plate; Standard wells: Take 2.5 μL of different concentrations of standards and add to the B wells of the plate; Sample wells: Take 2.5 μL of samples to be tested and add to the S wells of the plate.
2. Add 250 μL of reagent 1 to each well in step (1).
3. Incubate at 37°C for 10 min and measure the OD value using a microplate reader at a wavelength of 510 nm.

Note: When adding reagents to the plate wells, add them slowly to the bottom of the wells to avoid bubble formation (bubbles affect the measurement results).

The operation table is as follows:

	Blank Well	Standard Well	Sample Well
Anhydrous ethanol (μL)	2.5	--	--
Different concentration standards (μL)	--	2.5	--
Sample to be tested (μL)	--	--	2.5
Reagent 1 (μL)	250	250	250
Incubate at 37°C for 10 min and measure the OD value using a microplate reader at a wavelength of 510 nm.			

Calculation

The formula for calculating TC content in serum (plasma) and other liquid samples is: TC content (mmol/L) = $\frac{\Delta A1}{\Delta A2}$× c × f

The formula for calculating TC content in tissues is: TC content (mmol/kg wet weight) = $\frac{\Delta A1}{\Delta A2}$× c × f ÷$\frac{m}{V}$

ΔA1: Sample OD value - blank OD value

ΔA2: Standard OD value - blank OD value

c: Standard concentration (10 mmol/L)

f: dilution factor of the sample before adding it to the detection system

m: tissue sample mass (g)

V: volume of tissue sample homogenate (mL)

Notes

1. Reducing substances such as ascorbic acid, glutathione, etc. cannot be added to the sample.
2. The standard is an alcohol-soluble reagent that is volatile after opening. When operating a 96-well plate, try to add the standard after adding the sample, and add the working solution to the standard well first to reduce the volatility of the standard, thereby reducing the deviation.
3. The detection range of the kit is not equivalent to the concentration range of the analyte in the sample. If the concentration of the analyte in the sample is too high or too low, please dilute or concentrate the sample appropriately.
4. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.