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Ver.260801

Total Cholesterol Assay Kit

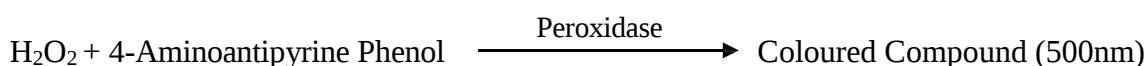
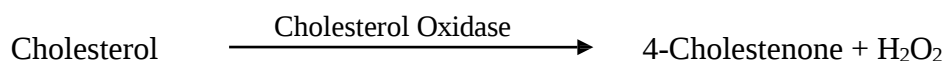
BC9908-02 (100Tests /96 Samples)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

Total cholesterol (TC) is the sum of cholesterol contained in all lipoproteins, including free cholesterol and cholesteryl esters.

The enzyme esterase catalyzes the hydrolysis of cholesteryl esters to produce free cholesterol (FC) and free fatty acids (FFA), thus converting cholesteryl esters to FC; furthermore, cholesteryl oxidase catalyzes the oxidation of FC to produce 4-Cholestenone and H₂O₂; finally, peroxidase catalyzes the oxidation of 4-aminoantipyrine and phenol by H₂O₂ to produce red quinones, which have a characteristic absorption peak at 500 nm. The colour shade is proportional to the TC content. Enzymatic colourimetric determination of total cholesterol according to the following reactions.



Kit components

Reagent	Volume	Storage
Reagent I	60mL	2-8°C
Reagent II	400μL	2-8°C
Reagent III	60μL	2-8°C
Standard	Powder	2-8°C
96-well plate	96 holes	-

Reagents and Equipment Required but Not Provided

Spectrophotometer/microplate reader, balance, low temperature table centrifuge, water-bath, micro glass cuvette/96 well flat-bottom plate, pipette, EP tube, distilled water, isopropyl alcohol.

Reagent Preparation

Extract: Isopropyl alcohol 110mL ×1. Required but not provided. Store at 2-8°C. A 30mL brown empty bottle is provided in the kit, which is only used for packaging. Please mark the name of the reagent yourself.

A handheld centrifuge to shake the liquid off to the bottom before use

Standard solution: Powder ×1. Store at 2-8°C. 10 mg cholesterol, add 517μL extract before use and shake to dissolve, 50μmol/mL cholesterol standard solution, the reagent can be stored at 2-8°C for 4 weeks.

Working solution: Prepare the working solution according to the sample volume of Reagent I: Reagent II: Reagent III in the ratio of 6mL: 40μL: 6μL (6.046mL, about 12T, when the solution will be used).

Operation Procedures

Sample Preparation

1. Bacteria or cells

According to the number of the cells (10^4): the volume of the extract (mL) is 500~1000:1. It is suggested that add 1mL of Extract to 500 million of cells. Breaking cells by ultrasonic wave in ice bath (power 300W, ultrasonic 2s, interval 3s, total time 3 min). Centrifuge at 10000g 4°C for 10 minutes. The supernatant should be kept on ice.

2. Tissue

According to the tissue mass (g) : the extract volume (mL) is 1:5-10. (It is recommended that add 1mL of extract to 0.1g tissue). Homogenate in ice bath, then centrifuge at 10000g for 10 minutes at 4°C. Take the supernatant for test.

3. Serum (Plasma) or Urine

Detect directly. If the solution has precipitate, please centrifuge and take the supernatant to be measured.

Procedure Notes

1. Preheat the spectrophotometer/microplate reader for more than 30 minutes, adjust the wavelength to 500nm and set the spectrophotometer counter to zero with distilled water for more than 30 minutes.
2. Preheat the Working solution at 37 °C for more than 10 minutes before use.
3. Dilute 50μmol/mL standard solution with Extract to 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625μmol/mL for standby.
4. Operation table: (Add the following reagents into 1.5mL centrifuge tube/96 well flat-bottom plate)

Reagent	Blank tube (A_B)	Standard tube (A_S)	Test tube (A_T)
Working Reagent	500μL	500μL	500μL
Standard	-	10μL	-
Sample	-	-	10μL
Extract	10μL	-	-

Mix thoroughly. Incubate at 37° C water bath or constant temperature incubator for 30 minutes. Use micro glass cuvette/96 well flat-bottom plate to measure the absorption value A at 500nm. Record as A_T , A_S , A_B . $\Delta A = A_T - A_B$, $\Delta A_S = A_S - A_B$. Each blank tube only need to test once or twice. **Note:** If the sample is serum, it is necessary to fill the serum blank tube (A_B'): change the extraction solution (isopropanol) to distilled water for the experiment. Calculate $A = A_T - A_B'$. Standard tube and Blank tube remain unchanged.

Calculation

1. Standard curve

According to the concentration of the standard tube (x , $\mu\text{mol/mL}$) and the absorbance ΔA s (y , ΔA s), a standard curve was established. According to the standard curve, ΔA (y , ΔA) was brought into the formula to calculate the sample concentration (x , $\mu\text{mol/mL}$).

2. Calculation of TC content

(1) Serum (plasma)

$$\text{TC content } (\mu\text{mol/dL}) = x \times 100 \times F$$

(2) Tissue

a. Calculate by protein concentration

$$\text{TC content } (\mu\text{mol/mg prot}) = x \times V_E \div (C_{pr} \times V_E) \times F = x \div C_{pr} \times F$$

b. Calculate by sample mass

$$\text{TC content } (\mu\text{mol/g fresh mass}) = x \times V_E \div W \times F = x \div W \times F$$

(3) Cells

$$\text{TC content } (\mu\text{mol}/10^6 \text{ cell}) = x \times V_E \div N \times F = x \div N \times F$$

100: 1 dL=100 mL

V_E : Extract volume, 1mL

W : Sample mass, g

500: The number of cells, 500 million

C_{pr} : The concentration of protein, mg/mL.

N : Total number of cells/bacteria, 10^6 cells as a unit.

F : Sample dilution multiple.

Note:

1. If the measured absorbance value exceeds the linear range absorbance value, you can increase the sample size or dilute the sample with the extraction solution and then perform the measurement. Note the simultaneous modification of the calculation formula.
2. The extraction solution contains components that denature the protein, so it is necessary to re-extract the protein for measurement when calculating by protein concentration.

Technical Specifications:

Minimum Detection Limit: $0.02 \mu\text{mol/mL}$

Linear Range: $0.03\text{-}15 \mu\text{mol/mL}$