



ISO 13485:2016 ISO 9001:2015

Ver. 240103

Total Cholesterol (TC) Assay Kit

BC9908-01 (50 Tests/48 Samples)

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

Product Description

Total cholesterol (TC) refers to the total cholesterol of all lipoproteins. It includes free cholesterol and cholesterol esters. Esterase can catalyse the hydrolysis of cholesterol ester to produce free cholesterol (FC) and free fatty acid (FFA), thus transforming cholesterol ester into FC. Furthermore, cholesterol oxidase can catalyse FC to form Δ^4 -cholesterone and H_2O_2 ; Finally, peroxidase can catalyse the oxidation of 4-aminoantipyrine and phenol by H_2O_2 to form red quinones. It has a characteristic absorption peak at 500 nm, and its colour intensity is directly proportional to TC content.

Kit components

Reagent	Volume	Storage
Extract Solution (Isopropyl Alcohol)	60mL \times 1 Not provided with the kit.	2-8°C
Reagent I	75mL \times 1	2-8°C
Reagent II	450 μ L \times 1	2-8°C
Reagent III	70 μ L \times 1	2-8°C
Standard	Powder \times 1 (10mg cholesterol.) Add 517 μ L Extract Solution before use and shake to dissolve. Final concentration is 50 μ mol/mL	2-8°C

Working Reagent

Mix Reagent I: Reagent II: Reagent III in the ratio 9mL : 60 μ L : 9 μ L.
This will be sufficient for 10 tests. Always prepare fresh.

Reagents and Equipment Required but Not Provided

Cooling centrifuge, spectrophotometer, 1mL glass cuvette and distilled water, isopropyl alcohol.

Protocol

I. Sample Preparation

Tissue: Prepare 10% tissue homogenate by adding 1mL Extract Solution to 0.1g tissue. Grind completely to make a homogenate. Centrifuge at 10000rpm, 4°C for 10 minutes and collect the supernatant.

Note: Ideal proportion of tissue weight (g) to Extract is 1 : 5 – 10.

Bacteria or cells: Harvest the cells and wash twice with PBS. Add 1ml Extract to 500 million cells and ultrasonicate (200W, work time 3 second/ interval 10 second repeat for 30 times) for complete lysis. Perform ultrasonication while keeping the cells in ice bath. Centrifuge at 10000g, 4°C for 10 minutes and collect the supernatant. The supernatant should be kept on ice.

Serum or Plasma

Directly use for the assay

II. Assay procedure

- Preheat the spectrophotometer reader/ microplate reader for 30 minutes, adjust wavelength to 500 nm and set zero with distilled water.
- Dilute 50 μ mol/mL standard solution with distilled water to 2, 1.25, 0.625, 0.3125, 0.15625, 0.078 μ mol/mL as working standards.

- Add reagents as mentioned in the list into 1.5mL centrifuge tube

Reagent	Test tube (T)	Standard tube (S)	Blank Tube (B)
Sample	100μL	-	-
Standard	-	100μL	-
Extract Solution	-	-	100μL
Working Reagent	900μL	900μL	900μL

- Mix thoroughly, place at 37°C water bath for 15minutes. Add to 1mL glass cuvette detect the absorbance value of each tube at 500 nm. Record absorbance as A_T and A_S , A_S
 $\Delta A = \Delta A_T - A_B$
 $\Delta A_S = A_S - A_B$

Note:

- **When ΔA is more than 1, diluted the sample with Extract and repeat the experiment.**
If the sample is serum, it is necessary to fill the serum blank tube (A_B): change the extract solution (isopropanol) to distilled water for the experiment. Calculate $A = A_T - A_B$. Standard tube and Blank tube remain unchanged.

Calculations

1. Standard curve

According to the concentration of the standard tube (x , μmol/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 , μmol/mL)

(1) Serum (plasma)

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

(2) Tissue

Calculate by protein concentration

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

Calculate by sample weight

$$\begin{aligned} \text{TC content } (\mu\text{mol/g fresh weight}) &= x \times V_E \div W \\ &= x \div W \end{aligned}$$

(3) Cell

$$\begin{aligned} \text{TC content } (\mu\text{mol}/10^4 \text{ cell}) &= x \times V_E \div 500 \\ &= 0.002x \end{aligned}$$

100:1 dL=100 mL

V_E : Extract volume, 1 mL;

W : Sample weight, g;

500: The number of cells, 500 million;

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

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. Formula for calculation also need to be modified accordingly.
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.