



Ver. 240116

Free Cholestrol (FC) Assay Kit

BC9909-01 (50 Tests/48 Samples)

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

Product Description

FC is the main component of cell membrane, and it is also an important raw material for the synthesis of adrenocortical hormone, sex hormone, bile acid and vitamin D. The concentration of FC can be used as an index of lipid metabolism. The determination principle: FC oxidase catalyzes FC to form 4-cholesterolenone and H_2O_2 , while the peroxidase catalyzes H_2O_2 , 4-aminoantipyrine and phenol to form red quinone compounds with an absorption peak at 500 nm, and the color depth is proportional to the content of FC.

Kit components

Reagent	Volume	Storage
Extract Solution	Self-Provided Reagent	RT
Reagent I	75 mL×1	2-8°C
Reagent II	450 μ L×1	2-8°C
Standard	Powder×1	2-8°C

Extract solution: Isopropanol (required but not provided. It takes about 60 mL), store at RT. A 30 mL brown empty bottle is provided in the kit, which is only used for packaging. Please mark the name of the reagent yourself.

Standard: Powder×1, 10 mg cholesterol, store at 2-8°C. Add 517 μ L isopropanol and prepare as 50 μ mol/mL cholesterol standard solution, then diluted to 1 μ mol/mL standard with isopropanol for test. The unused reagent can be stored at 2-8°C for 4 weeks.

Working solution: According to the sample number, the working solution is prepared according to the ratio of Reagent 1 : Reagent 2 =9mL : 60 μ L (about 10T).

Reagents and Equipment Required but Not Provided

Water bath, pipettes, spectrophotometer, 1 mL glass cuvette, mortar/homogenizer/cell ultrasonic crusher, absolute ethanol, distilled water

Protocol

I. Sample Preparation

Tissue: Accordance ratio weight (g): Extract solution(mL)=1: 5~10. (Add 1 mL Extract solution to 0.1 g tissue). Homogenate on ice bath. Centrifuge at $8000 \times g$ for 10 minutes at 4°C. Take supernatant for test.

Bacteria or fungus: Accordance ratio cell amount (10^4): Extract solution(mL)=500~1000:1. (Suggest 5 million with 1 mL Extract solution). Break cells (power 300w, work time 2seconds, interval 3seconds for 3 minutes) by ultrasonic on ice bath. $8000 \times g$ centrifuge for 10 minutes at 4°C. Take supernatant for test.

Serum or Plasma

Detect directly.

II. Determination procedure.

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 500 nm, set zero with distilled water.
2. Take out certain quantity working solution, preheat at 37°C for 30 minutes. The rest store at 2-8°C.
3. Dilution of standard solution: 50µmol/mL cholesterol standard solution is diluted with extract solution to obtain 1.25, 0.625, 0.3125, 0.15625, 0.078125, 0.039, 0.0195µmol/mL standard substance for later use.
4. Standard dilution can refer to the following table.

Sl.No.	Pre-dilution concentration (µmol/mL)	Standard solution volume (µL)	Extract solution volume (µL)	Concentration after dilution (µmol/mL)
1	50	25	975	1.25
2	1.25	500	500	0.625
3	0.625	500	500	0.3125
4	0.3125	500	500	0.15625
5	0.15625	500	500	0.078125
6	0.078125	500	500	0.039
7	0.039	500	500	0.0195

5. Add reagents according to the following table.

Reagent Name:	Blank tube (A _B)	Standard tube (A _S)	Test tube (A _T)
Sample	100	-	-
Standard	-	100	-
Isopropanol	-	-	100
Working solution	900	900	900

Fully mixed, stand at 37 °C for 30 minutes. After the reaction completed, the absorbance value A at 500 nm is measured in 1 mL glass cuvette,

which is recorded as A_T, A_S and A_B, $\Delta A_T = A_T - A_B$, $\Delta A_S = A_S - A_B$. Blank tube and standard curve only need to measure 1-2 times.

Note: If the sample is a liquid sample such as serum (plasma), it is necessary to add a 'serum (plasma) blank tube' - the extract (isopropanol) in the blank tube is replaced with distilled water for the experiment, and the calculation of $\Delta A_T = A_T - A_{B-serum (plasma)}$, standard tube determination and ΔA_S calculation remain unchanged.

Calculations

I. 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 , $\mu\text{mol/mL}$).

II. Calculate of TC content

(1) Serum (plasma)

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

(2) Tissue

- Calculate by protein concentration

$$\text{TC content } (\mu\text{mol/mg prot}) = x \times \text{VE} \div (\text{Cpr} \times \text{VE}) = x \div \text{Cpr}$$

- Calculate by sample weight

$$\text{TC content } (\mu\text{mol/g fresh weight}) = x \times \text{VE} \div W = x \div W$$

(3) Cells

$$\text{TC content } (\mu\text{mol}/10^4 \text{ cell}) = x \times \text{VE} \div 500 = 0.002x$$

100:1 dL=100 mL

VE: Extract volume, 1 mL;

W: Sample weight, g;

500: The number of cells, 500 million;

Cpr: 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. 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 : 0.056 $\mu\text{mol/mL}$

Linear Range : 0.078-2 $\mu\text{mol/mL}$