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

Catalog Number: K086

Size: 48T(44S)/ 96T(92S)

Detection instrument: Microplate reader(495-525 nm)

Species: Universal

Application: Quantitative detection of total cholesterol (TC) content in serum, plasma, tissue samples.

Storage Conditions: 2-8°C for 6 months

Shelf life: See kit label

Note: For research use only.

Reagent Components:

Item	Component	Size 1 48T	Size2 96T	Storage
Reagent 1	Enzyme Solution	15mLx1 vial	30 mLx1 vial	2-8°C, 6 months shading light
Reagent 2	5.17 mM Cholesterol Standard	0.2 mLx1 vial	0.2 mLx1 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. For a small volume of reagents, please centrifuge before use, so as to obtain sufficient amount of reagents.

Assay Principle:

Total cholesterol(TC) includes free cholesterol and cholesterol esters. Cholesterol ester can be hydrolyzed by cholesterol esterase(CE) to produce cholesterol and free fatty acid. Cholesterol is oxidized by cholesterol oxidase to produce △4-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.

Additional Materials Required:

1. Microplate reader(495-525 nm)
2. 96-well plate
3. Precision Micropipettor and clean disposable tips
4. Clean EP tubes
5. absolute ethanol, Normal saline (0.9% NaCl), Double distilled water
6. Benchtop centrifuge
7. Constant temperature water bath/incubator

Sample Preparation:

Sample requirements: No reducing substances, such as ascorbic acid and glutathione, can be added to the sample.

Serum and plasma: detect directly.

Tissue sample: According to the tissue mass (g) : absolute ethanol volume (mL) 1:5 ~10 ratio (it is recommended to weigh about 0.1g tissue, add 1mL absolute ethanol), the mixture is homogenized in ice bath. 10000g, centrifuge at 4℃ for 10min, and take the supernatant on ice to be measured.

Before the formal test, 2-3 samples with significant differences should be selected and diluted into different concentrations for pre-test. According to the results of the pre-experiment and the linear range of this kit: 0.29-25.85 mmol/L, the recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor	Sample type	Dilution factor
Human serum	No Dilution	Mouse serum	No Dilution
Rat serum	No Dilution	10% mouse kidney tissue	No Dilution

Note: The diluent of serum(plasma) is normal saline (0.9% NaCl). The diluent of tissue Sample is absolute ethanol.

Reagent preparation:

Equilibrate all reagents to room temperature before use.

Assay Procedure:

1. Standard well: Take 2.5 μL of standard to the wells.

   Sample well: Take 2.5 μL of sample to the wells.

   Blank well: Take 2.5 μL of double-distilled water to the wells.

2. Add 250 μL of Enzyme Solution to each well and mix fully.

3. Cover the plate sealer and incubate at 37 °C for 15 min. Measure the OD value of each well with microplate reader at 510 nm.

Operation table:

	Blank well	**Standard well**	**Sample well**
double-distilled water(μL)	2.5		
5.17 mM Cholesterol Standard(μL)		2.5	
sample to be tested(μL)			2.5
enzyme solution(μL)	250	250	250
Incubate at 37 °C for 15 min. Measure the OD value of each well with microplate reader at 510 nm.			

Calculation:

1. Serum (plasma) sample and other liquid samples:

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

2. Tissue sample:

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

Note:

ΔA1: OD_{sample}-OD_{blank}

ΔA2: $OD_{standard}$-OD_{blank}

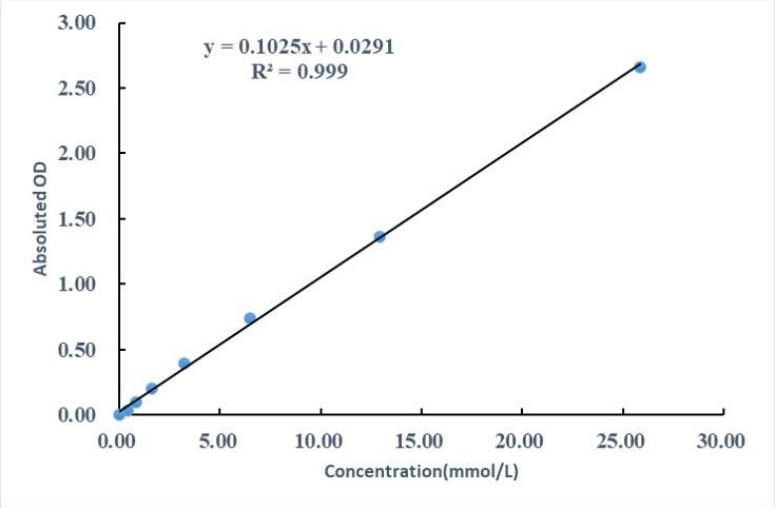
f: Dilution factor of sample before tested.

c: the concentration of standard, 5.17 mmol/L.

m: the weight of tissue sample, g.

V: the volume of the homogenate of tissue samples, mL.

Standard curve: (The standard curve is provided as below for reference only.)



Note:

1. This assay kit is for Research Use Only. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments. Protection methods must be taken by wearing lab coat and latex gloves.

2. The detection range of the kit is not equivalent to the concentration range of the substance to be measured in the sample. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample. It is recommended to take a pre-test if your sample is not listed in the instruction book.

3. The experimental results are closely related to the situation of reagents, operations, environment and so on. Fine Test will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.