# Technical Manual

# Total Cholesterol (TC) Colorimetric Assay Kit (Single Reagent, COD-PAP Method)

- Catalogue Code: MAES0093
- Size: 100 Assays
- Research Use Only

# 1. Key features and Sample Types

## Detection method:

Colorimetric method

## Specification:

100Assays

## Range:

0.09-25.85 mmol/L

## Sensitivity:

0.09 mmol/L

## Storage:

2-8°C for 6 months

## Expiry:

See Kit Label

## Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

## 2. Background

Cholesterol is a kind of sterol and lipid in cell membrane. Most of cholesterol in blood exists in the form of cholesterol ester. Lecithin-cholesterol acyltransferase in human plasma is an enzyme that catalyzes the formation of cholesterol ester. Cholesterol synthesized or deposited in peripheral cells returns to the liver through the reverse cholesterol transport system for reusing or regaining bile acids.

## 3. Intended Use

This kit applies the COD-PAP method and it can be used for in vitro determination of total cholesterol (TC) content in serum, plasma, animal tissue and cells samples.

## 4. Detection Principle

Total cholesterol includes free cholesterol and cholesterol esters. Cholesterol ester can be hydrolyzed by cholesterol esterase 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. The absorbance values of the standard tube and the sample tube are measured respectively, and the cholesterol content in the sample can be calculated.

## 5. Kit components & storage

| Item | Specification | Storage |
|---|---|---|
| **Enzyme Working Solution** | 60 mL × 2 vials | 2-8°C, 6 months, avoid direct sunlight |
| **Cholesterol Standard (5.17 mM)** | 0.5 mL × 1 vial | 2-8°C, 6 months |
| **Microplate** | 96 wells | No requirement |
| **Plate Sealer** | 2 pieces | |

### Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Spectrophotometer (510 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)
- Isopropanol

## 6. Assay Notes:

1. Since the volume of standard and sample is 10 μL, it is necessary to adhere to the wall of the EP tubes when adding the liquid to reduce the error.

2. When measuring low content samples such as cells, the volume of sample should be increased to 20 μL, and the volume of blank well and standard well should be increa sed at the same time.

## 7. Reagent preparation:

Bring all reagents to room temperature before use.

## 8. Sample Preparation

**Sample requirements**

Reducing substances such as ascorbic acid and glutathione should not be added to the sample.

**1. Serum sample:**

Collect fresh blood and stand at 25°C for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80°C for a month.

**2. Plasma sample:**

Take fresh blood into the tube which has anticoagulant, centrifuge at 700-1000 g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

**3. Cell sample:**

Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number ($2\times10^6$): Isopropanol (μL) =1: 200. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

**4. Tissue sample:**

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of Isopropanol (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant to preserve it on ice for detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

### Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

### Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.09-25.85 mmol/L).

The recommended dilution factor for different samples is as follows (for reference only).

| Sample Type: | Dilution Factor |
|---|---|
| Human serum | 1 |
| Mouse serum | 1 |
| Rat plasma | 1 |
| 10% Mouse liver tissue homogenate | 1 |
| 10% Mouse kidney tissue homogenate | 1 |
| 10% Mouse heart tissue homogenate | 1 |
| HepG2 cells | 1 |

**Note:**The diluent of serum (plasma) is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7. 4); The diluent of animal tissue and cells is isopropanol.

## 9. Assay Protocol

**Ambient Temperature:** 25-30°C

**Optimum detection wavelength:** 510 nm

## 10. Operation Steps

1. **Blank tube:** add 10 μL of double distilled water to 2 mL EP tube.
   **Standard tube:** add 10 μL of 5.17 mM cholesterol standard to 2 mL EP tube.
   **Sample tube:** add 10 μL of sample to 2 mL EP tube.

2. Add 1000 μL of enzyme working solution to each tube.

3. Mix thoroughly, incubate at 37°C for 10 min. Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 550 nm with 0.5 cm diameter cuvette.

### Operation Table

| | Blank tube | Standard tube | Sample tube |
|---|---|---|---|
| **Double distilled water (μL)** | 10 | | |
| **5.17 mM Cholesterol standard (μL)** | | 10 | |
| **Sample (μL)** | | | 10 |
| **Enzyme working solution (μL)** | 1000 | 1000 | 1000 |
| Mix thoroughly and incubate at 37°C for 10 min. Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 510 nm with 0.5 cm optical path quartz cuvette. | | | |

## 11. Calculations

**1. Serum (plasma) sample:**

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

**2. Tissue sample:**

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

**3. Cell sample:**

$$\text{TC content (μmol/10}^6\text{ cells)} = \frac{\Delta A_1}{\Delta A_2} \times c \div \frac{N}{V} \times f$$

**$\Delta A_1$:** $OD_{sample}$-$OD_{blank}$
**$\Delta A_2$:** $OD_{standard}$-$OD_{blank}$
**c:** The concentration of standard, 5.17 mmol/L
**f:** Dilution factor of sample before tested
**m:** The weight of tissue sample, mg
**V:** The volume of isopropanol, mL
**N:** The number of cells. For example, the number of cells is $5*10^6$, N is 5



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## 12. Performance Characteristics

| | |
|---|---|
| **Detection Range** | 0.09-25.85 mmol/L |
| **Sensitivity** | 0.09 mmol/L |
| **Average recovery rate (%)** | 102 |
| **Average inter-assay CV (%)** | 2.8 |
| **Average intra-assay CV (%)** | 1.1 |

### Analysis

Take 10 μL of human serum, carry the assay according to the operation table.

**The results are as follows:**
The average OD value of the sample is 0.186, the average OD value of the blank is 0.028, the average OD value of the standard is 0.210, and the calculation result is:

$$\text{TC content (mmol/L)} = \frac{0.186-0.028}{0.210-0.028} \times 5.17 = 4.49 \text{ (mmol/L)}$$

## Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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