TECHNICAL MANUAL

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

- **SKU CODE:** MAES0375
- **SIZE:** 100 Assays
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

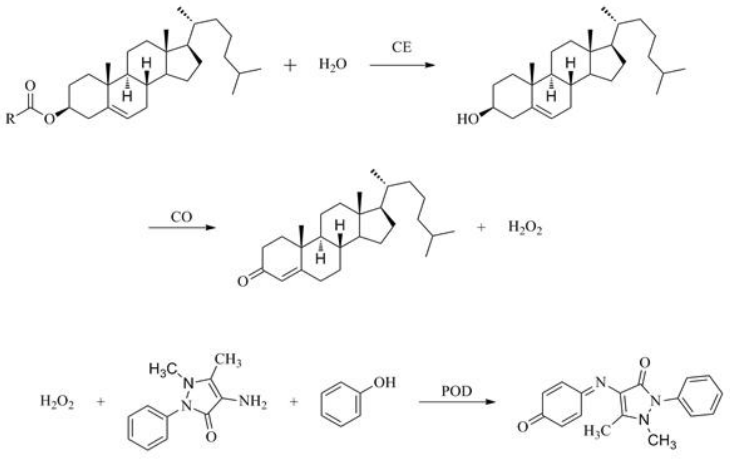
- Reagent preparation
- Sample preparation
- Add samples and standards to tubes
- Add enzyme working solution
- Incubate and measure absorbance

2. Intended use

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

3. Detection principle

Total cholesterol includes free cholesterol and cholesterol esters. Cholesterol ester is hydrolyzed by cholesterol esterase to produce cholesterol and free fatty acid. Cholesterol is oxidized by cholesterol oxidase to produce Δ4-cholestenone and hydrogen peroxide. In the presence of 4-aminoantipyrine and phenol, hydrogen peroxide catalyzes peroxidase to form red quinone compounds of benzoquinone imine phenazone. The color intensity 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.



4. Kit components & storage

Item	Component	Size 1 (50 Assays)	Size 2 (100 Assays)	Storage
Reagent 1	Enzyme Working Solution	60 mL x 1 vial	60 mL x 2 vials	2-8°C, 12 months, protect from light
Reagent 2	5.17 mM Cholesterol Standard	0.25 mL x 1 vial	0.5 mL x 1 vial	2-8°C, 12 months

5. Materials prepared by users

Instruments:

Spectrophotometer (510 nm), Micropipettor, Incubator, Centrifuge

Reagents:

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4), Anhydrous ethanol.

Note: The reagents must be stored strictly according to the preservation conditions in the table. The reagents from different kits cannot be mixed with each other. For small volume reagents, please centrifuge before use to obtain sufficient reagent amount.

6. Reagent preparation

Equilibrate all reagents to room temperature before use.

7. Sample preparation

1. **Sample preparation:** Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for one month. Tissue sample: 1. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg). 2. Wash tissue in cold PBS (0.01 M, pH 7.4). 3. Homogenize 20 mg tissue in 180 µL anhydrous ethanol with a dounce homogenizer at 4°C. 4. Centrifuge at 10,000xg for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.
2. **Sample dilution:** The recommended dilution factor for different samples is as follows (for reference only): 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 Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). The diluent for animal tissue is anhydrous ethanol. For

dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. Key points of the assay

1. Protect the reagent from contamination with glucose, cholesterol, etc.
2. 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 error.
3. When measuring low-content samples, the volume of sample should be increased to 20 μL, and the volume of blank well and standard well should be increased at the same time.

9. Operating 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 and mix thoroughly.
3. 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 diameter cuvette.

10. Calculation

The sample:

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

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

2. Tissue sample:

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

[Note]

ΔA_1: OD_{sample} - OD_{blank}

ΔA_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, g.

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

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	1.50	9.70	19.50
%CV	1.3	1.0	1.0

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	1.50	9.70	19.50
%CV	2.5	2.8	3.1

Recovery

Three samples of high concentration, middle concentration, and low concentration were tested with 6 parallel measurements for each concentration to obtain an average recovery rate of 100%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mmol/L)	3.6	11.7	23.5

	Sample 1	Sample 2	Sample 3
Observed Conc. (mmol/L)	3.8	11.5	24.2
Recovery rate (%)	105	98	103

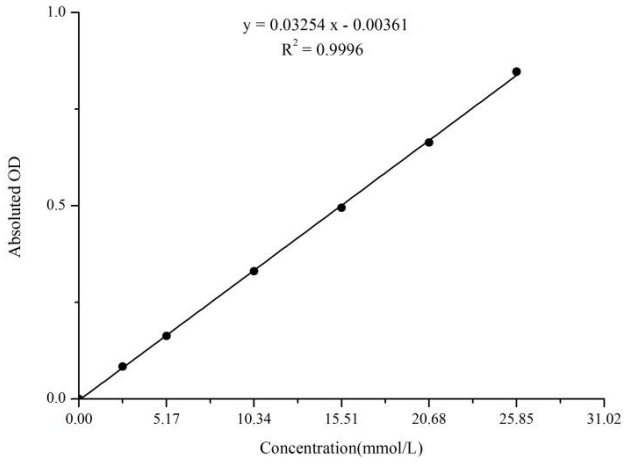
Sensitivity

The analytical sensitivity of the assay is 0.09 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

(It is not necessary to prepare the standard curve for this kit and the provided standard curve is for reference only)

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), the standard curve and data are provided below for reference only:



Concentration (mmol/L)	OD value		Average OD	Absolute OD
0	0.008	0.006	0.007	0
2.58	0.090	0.092	0.091	0.084

Concentration (mmol/L)	OD value		Average OD	Absolute OD
5.17	0.172	0.168	0.170	0.163
10.34	0.335	0.341	0.338	0.331
15.51	0.503	0.501	0.502	0.495
20.68	0.671	0.671	0.671	0.664
25.85	0.855	0.853	0.854	0.847

12. Appendix II Example Analysis

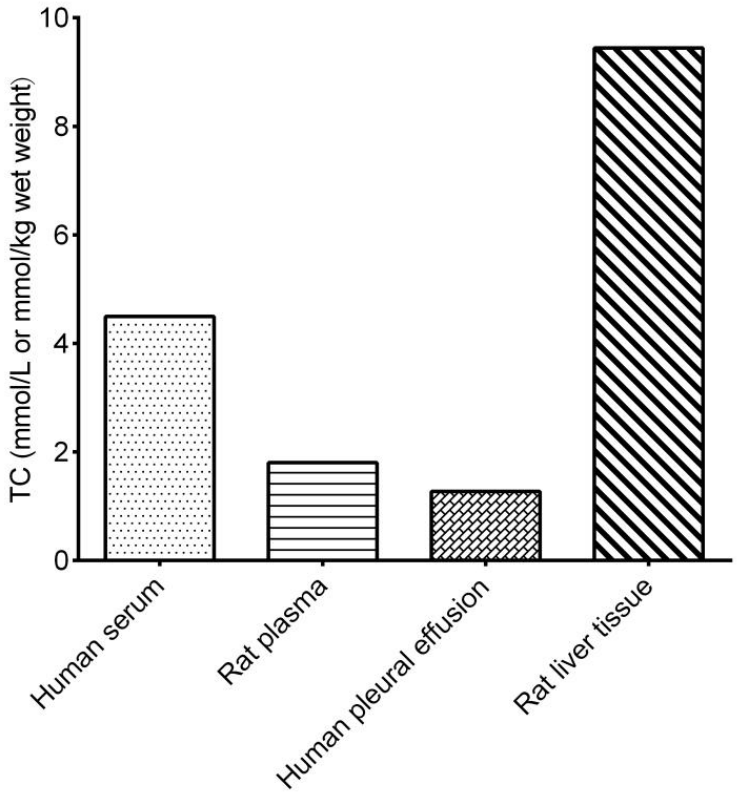
Example analysis:

Take 10 μL of human serum, perform the assay according to the operation steps. 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:

TC content (mmol/L) = (0.186 - 0.028)/(0.210 - 0.028) × 5.17 = 4.49 (mmol/L)

Detect human serum, rat plasma, human pleural effusion and rat liver tissue homogenate according to the protocol, the results are as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Please follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use

of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.