TECHNICAL MANUAL

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

- **SKU CODE:** MAES0093
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Sample preparation
- Add sample and reagents
- Incubate
- 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.

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 hydrogen peroxide. In the presence of Trinder's reagent, hydrogen peroxide is catalyzed by peroxidase (POD) to produce a red quinoneimine dye, whose color intensity is directly proportional to the TC concentration and can be measured at 510 nm.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Size 3(500 Assays)	Storage
Reagent 1	Enzyme Working Solution	15 mL x 1 vial	30 mL x 1 vial	50 mL x 3 vials	2-8 °C, 12 months, shading light
Reagent 2	5.17 mM Cholesterol Standard	0.2 mL x 1 vial	0.2 mL x 1 vial	1 mL x 1 vial	2-8 °C, 12 months
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces			

5. Materials prepared by users

Instruments:

Microplate reader (495-525 nm, optimum wavelength: 510 nm), Micropipettor, Incubator, Centrifuge

Reagents:

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

6. Reagent preparation

Equilibrate all reagents to room temperature before use.

7. Sample preparation

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 a 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 10,000 xg for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

Dilution of sample:

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) for serum and plasma; The diluent is anhydrous ethanol for tissue samples. For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. Avoid bubble formation when dispensing reagents into the microplate.
2. Protect the reagent from contamination by glucose, cholesterol, and other interfering substances.

9. Operating steps

1. Blank well: Add 2.5 μL of double distilled water to the corresponding wells. Standard well: Add 2.5 μL of 5.17 mM cholesterol standard to the corresponding wells. Sample well: Add 2.5 μL of sample to the corresponding wells.
2. Add 250 μL of enzyme working solution to each well.
3. Mix thoroughly, incubate at 37 °C for 10 min, measure the OD value at 510 nm with microplate reader.

10. Calculation

The sample:

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

TC content (mmol/L) = (ΔA_1 / ΔA_2) × c × f

2. Tissue sample:

TC content (mmol/kg wet weight) = (ΔA_1 / ΔA_2) × c × f ÷ (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)

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	2.50	16.80	22.00
%CV	3.3	3.2	2.8

Inter-assay Precision

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

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	2.50	16.80	22.00
%CV	8.0	8.3	8.6

Recovery

Three samples of high, middle, and low concentrations were tested in 6 parallel replicates for each concentration to obtain an average recovery rate of 103%.

Recovery

Sample 1	Sample 2	Sample 3
4.5	16.7	20.5
4.6	17.5	20.9
102	105	102

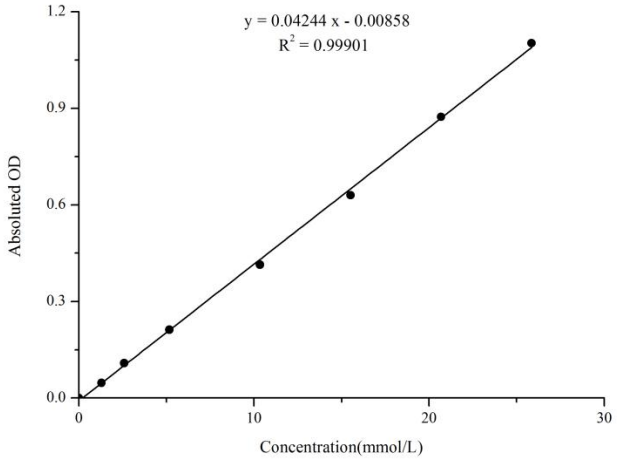
Sensitivity

The analytical sensitivity of the assay is 0.29 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; 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:



Standard curve data

Concentration (mmol/L)	OD value		Average OD	Absolute OD
0	0.054	0.053	0.054	0
1.29	0.100	0.102	0.101	0.047
2.58	0.160	0.166	0.163	0.109
5.17	0.267	0.265	0.266	0.212
10.34	0.467	0.469	0.468	0.414

Concentration (mmol/L)	OD value		Average OD	Absolute OD
15.51	0.685	0.683	0.684	0.630
20.68	0.928	0.928	0.928	0.874
25.85	1.155	1.159	1.157	1.103

12. Appendix II Example Analysis

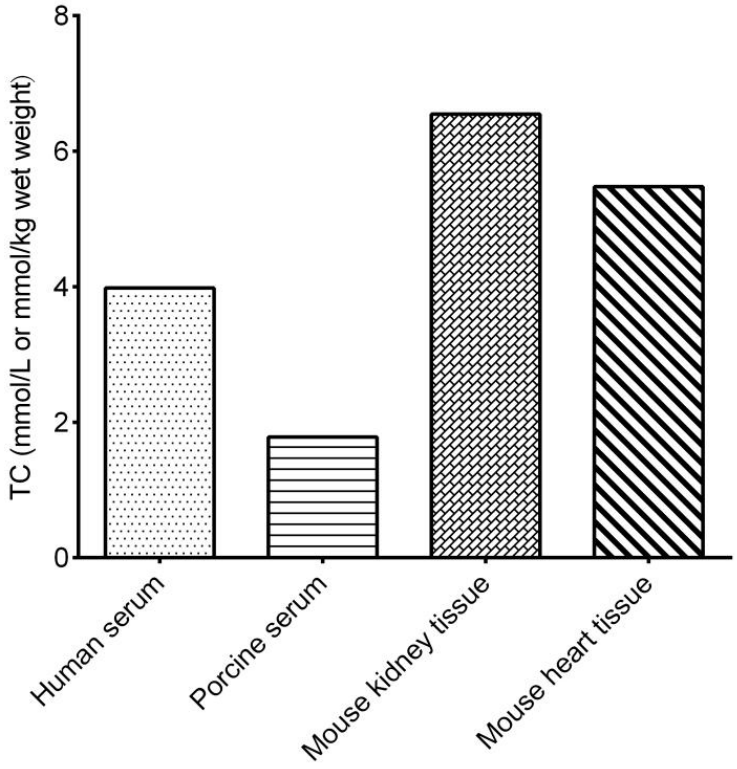
Example analysis:

Take 2.5 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 0.239, the average OD value of the blank is 0.049, the average OD value of the standard is 0.295, and the calculation result is:

Total Cholesterol content (mmol/L) = (0.239-0.049)/(0.295-0.049) × 5.17 × 1 = 3.99 mmol/L

Detect human serum, porcine serum, 10% mouse kidney tissue homogenate, 10% mouse heart tissue homogenate according to the protocol. The result is 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. 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 pretest 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 calculate 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.