TECHNICAL MANUAL

Free Cholesterol (FC) Colorimetric Assay Kit

- **SKU CODE:** MAES0017
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

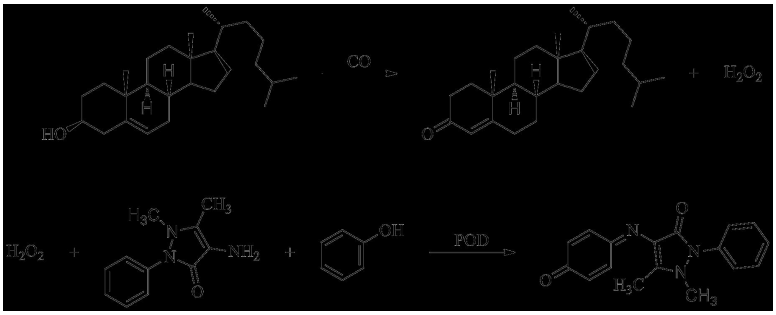
Revised: 01/2026

1. Intended use

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

2. Detection principle

Free cholesterol produces 4-cholestenone and hydrogen peroxide under the oxidation of cholesterol oxidase. In the presence of 4-aminoantipyrine and phenol, peroxidase catalyzes hydrogen peroxide to form red quinone compounds of benzoquinone imine phenazone. The color depth of the generated quinones is directly proportional to the cholesterol content.



3. 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, protect from light
Reagent 2	5.17 mM Cholesterol Standard	0.2 mL x 1 vial	0.2 mL x 1 vial	0.5 mL x 1 vial	2-8°C, 12 months
	Microplate	96 wells	/	/	No requirement
	Plate Sealer	2 pieces			

4. Materials prepared by users

Instruments:

Microplate reader (500-520 nm, optimum wavelength: 510 nm), Micropipette, Vortex mixer, Incubator, Centrifuge

Reagents:

Double distilled water, Normal saline (0.85% NaCl), PBS (0.01 M, pH 7.4), Anhydrous ethanol

5. Reagent preparation

Equilibrate all reagents to room temperature before use.

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

6. Sample preparation

1. **Sample preparation:** Serum and plasma: detect directly. If not detected on the same day, 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 10,000 x g for 10 min to remove insoluble material. Collect supernatant and keep on ice for detection. ⑤ Meanwhile, determine the protein concentration of supernatant (MAES0177).
2. **Sample dilution:** The recommended dilution factor for different samples is as follows (for reference only): Human plasma: 1 Human serum: 1 Rat serum: 1 Mouse serum: 1 Rabbit serum: 1 10% Rat kidney tissue homogenate: 1 Note: The diluent is normal saline (0.85% NaCl) or PBS (0.01 M, pH 7.4). For dilution of other sample types, please perform a pretest to confirm the dilution factor.

7. Key points of the assay

1. Prevent bubble formation when adding reagents to the microplate.
2. Equilibrate all reagents to room temperature before use.

8. Operating steps

1. Blank well: add 5 µL of double distilled water into the well. Standard well: add 5 µL of standard solution into the well. Sample well: add 5 µL of sample into the well.

2. Add 250 µL of enzyme working solution to each well.

3. Mix thoroughly, incubate at 37°C for 10 min, measure absorbance value at 510 nm with microplate reader.

9. Calculation

The sample:

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

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

2. Tissue sample:

Free cholesterol 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 anhydrous ethanol, mL

10. 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)	1.00	10.00	17.00
%CV	2.5	1.9	1.3

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)	1.00	10.00	17.00
%CV	5.7	4.3	5.6

Recovery

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

Recovery

	Sample 1	Sample 2	Sample 3
Expected Conc. (mmol/L)	8	18	20
Observed Conc. (mmol/L)	7.9	17.6	18.8
Recovery rate (%)	99	98	94

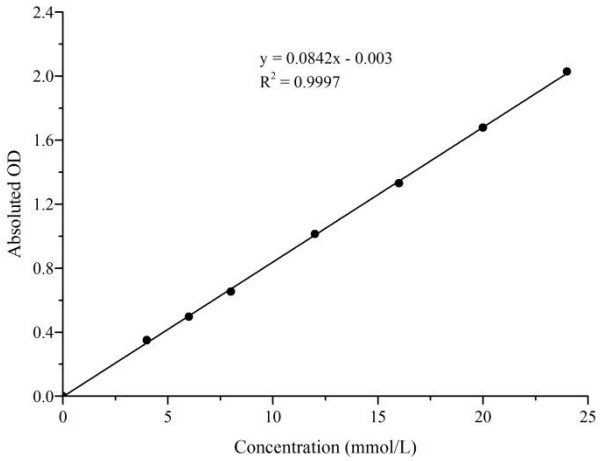
Sensitivity

The analytical sensitivity of the assay is 0.07 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 1	OD value 2	Average OD	Absolute OD
0	0.078	0.077	0.078	0.000
4	0.420	0.436	0.428	0.351
6	0.567	0.581	0.574	0.497
8	0.734	0.728	0.731	0.653
12	1.082	1.102	1.092	1.014
16	1.380	1.436	1.408	1.331
20	1.767	1.746	1.756	1.679
24	2.122	2.092	2.107	2.029

11. Appendix II Example Analysis

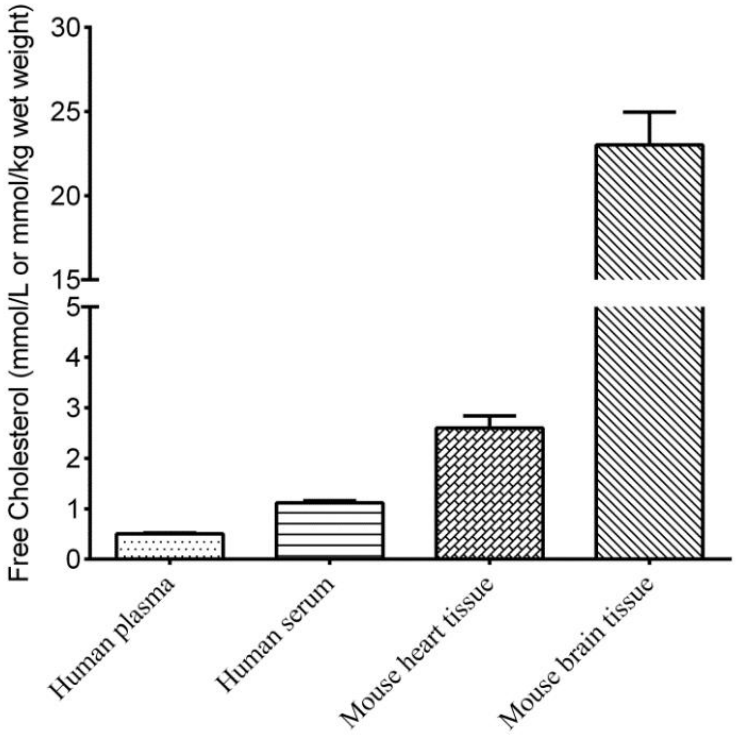
Example analysis:

For mouse brain tissue, take 5 μL of 10% mouse brain tissue homogenate and carry out the assay according to the operating steps. The results are as follows:

The average OD value of the sample is 0.318, the average OD value of the blank is 0.089, the average OD value of the standard is 0.519, and the calculation result is:

Free cholesterol content (mmol/kg wet weight) = (0.318-0.089) ÷ (0.519-0.089) × 5.17 × 1 ÷ 0.02/0.18 = 24.78 mmol/kg wet weight

Detect human plasma, human serum, 10% mouse heart tissue homogenate, and 10% mouse brain tissue homogenate according to the protocol. The results are as follows:



12. Statement

1. This assay kit is for Research Use Only. Assay Genie will not be responsible for any problems or legal liabilities arising from using the kit for clinical diagnosis or any other purpose.

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

3. Protective measures must be taken by wearing a lab coat and latex gloves.

4. If the concentration of the substance is not within the detection range exactly, an additional dilution or concentration should be performed on the sample.

5. It is recommended to perform a pretest if your sample is not listed in the instruction manual.

6. The experimental results are closely related to reagent conditions, operations, environment, and other factors. Assay Genie will guarantee the quality of the kits

only and will NOT be responsible for sample consumption caused by using the assay kits. It is advisable to calculate the possible sample usage and reserve sufficient samples before use.

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.