TECHNICAL MANUAL

Low-Density Lipoprotein Cholesterol (LDL-C) Colorimetric Assay Kit

- **SKU CODE:** MAES0408
- **SIZE:** 100 Assays
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Intended use

This kit can be used to measure low-density lipoprotein cholesterol (LDL-C) content in serum (plasma) and animal tissue samples.

2. Detection principle

Lipoproteins (except low-density lipoprotein cholesterol (LDL)) such as high-density lipoprotein (HDL), chylomicron (CM), and very low-density lipoprotein (VLDL) change structure and dissociate under the action of surfactants. The released micronized cholesterol molecules react with cholesterol enzyme reagents, and the generated hydrogen peroxide is trapped in the absence of coupling agent. It is consumed without color development.

At this time, the LDL particles remain intact, and then the reagent containing coupling agent is added, which can dissociate the LDL particles to release cholesterol. This cholesterol is catalyzed by cholesterol esterase (CE) and cholesterol oxidase (CO) to produce hydrogen peroxide. Hydrogen peroxide is catalyzed by peroxidase (POD) in the presence of 4-aminoantipyrine (4-AA) and phenol (T-OOS) to form a red quinone compound. The intensity of the color is directly proportional to the LDL-C content.

3. Kit components & storage

Item	Component	Size 1 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Reaction Solution	45 mL × 1 vial	45 mL × 2 vials	2-8 °C, 12 months, protected from light
Reagent 2	Working Solution	14 mL × 1 vial	28 mL × 1 vial	2-8 °C, 12 months, protected from light
Reagent 3	Standard	Powder × 2 vials	Powder × 4 vials	2-8 °C, 12 months, protected from light

4. Materials prepared by users

Instruments:

Spectrophotometer (546 nm), Vortex mixer, Incubator

Reagents:

Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4), Isopropyl alcohol (AR)

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 small volume reagents, please centrifuge before use to ensure sufficient reagent is obtained.

5. Reagent preparation

1. Equilibrate all reagents to 25 °C before use.
2. **Preparation of standard solution:** Dissolve one vial of standard with 200 µL of double distilled water, mix well to dissolve. Store at 2-8 °C for 2 weeks protected from light.

6. 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 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 isopropyl alcohol with a dounce homogenizer at 4 °C. 4. Centrifuge at 10000×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.
2. **Dilution of sample:** The recommended dilution factor for different samples is as follows (for reference only): Sample type - Dilution factor Human serum - 1 Human plasma - 1 Mouse serum - 1 Mouse plasma - 1 Rat serum - 1 Rat plasma - 1 Porcine serum - 1 Rabbit serum - 1 Horse serum - 1 10% Mouse lung tissue homogenate - 1 10% Mouse kidney tissue homogenate - 1 10% Mouse liver tissue homogenate - 1 Note: The diluent for serum (plasma) is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). The diluent for animal tissue is isopropyl alcohol. For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

7. Operating steps

1. Before detection, incubate all reagents in a 25 °C incubator for 15 min in advance.
2. Blank tube: Take 20 µL of double distilled water to the EP tubes. Standard tube: Take 20 µL of standard solution to the EP tubes. Sample tube: Take 20 µL of sample to the EP tubes.

3. Add 720 µL of reaction solution to each tube.
4. Mix fully by vortex mixer and incubate at 37 °C for 5 min protected from light. Set the spectrophotometer to zero with double distilled water and measure the absorbance at 546 nm with 1 cm optical path quartz cuvette, as A1.
5. Add 240 µL of working solution to each tube.
6. Mix fully by vortex mixer and incubate at 37 °C for 5 min protected from light. Set the spectrophotometer to zero with double distilled water and measure the absorbance at 546 nm with 1 cm optical path quartz cuvette, as A2.

8. Calculation

The sample:

1. Serum (plasma) sample:

LDL-C content (mmol/L) = ΔA1/ΔA2 × c × f

2. Tissue sample:

LDL-C content (mmol/kg wet weight) = ΔA1/ΔA2 × c × f ÷ m/V

[Note]

ΔA1: $\triangle A_{sample}$ - $\triangle A_{blank}$. ΔA = A_2 - A_1.

ΔA2: $\triangle A_{standard}$ - $\triangle A_{blank}$. ΔA = A_2 - A_1.

c: Concentration of standard. Its concentration is shown on the label, mmol/L.

f: Dilution factor of sample before tested.

m: The weight of the sample, g.

V: The volume of isopropyl alcohol in the preparation step of sample, mL.

9. 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	1.20	2.00

Parameters	Sample 1	Sample 2	Sample 3
%CV	1.1	4.8	1.6

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	1.20	2.00
%CV	3.2	7.3	5.1

Sensitivity

The analytical sensitivity of the assay is 0.061 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.

10. Appendix II Example Analysis

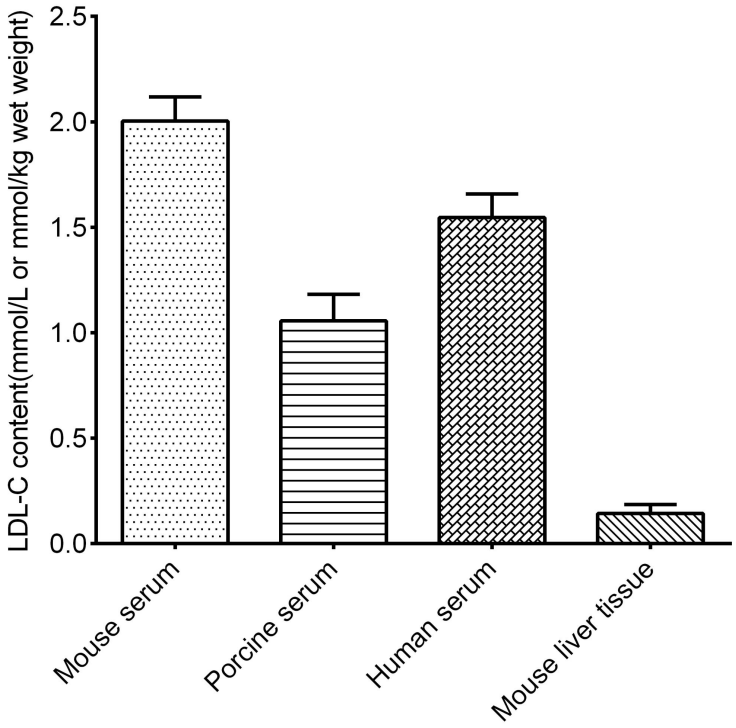
Example analysis:

Take 20 µL of human serum and perform the assay according to the operation steps. The results are as follows:

The A_1 of blank is 0.009, the A_2 of blank is 0.013, $\Delta A_{blank} = 0.013 – 0.009 = 0.004$. The A_1 of standard is 0.087, the A_2 of standard is 0.351, $\Delta A_{standard} = 0.351 – 0.087 = 0.264$. The A_1 of sample is 0.050, the A_2 of sample is 0.229, $\Delta A_{sample} = 0.229 – 0.050 = 0.179$, the concentration of standard is 2.3 mmol/L, and the calculation result is:

LDL-C (mmol/L) = (0.179 – 0.004) ÷ (0.264 – 0.004) × 2.3 = 1.55 mmol/L

Detection of mouse serum, porcine serum, human serum and 10% mouse liver tissue homogenate, according to the protocol, the result is as follows:



11. 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 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.