Technical Manual

Low-density Lipoprotein Cholesterol (LDL-C) Colorimetric Assay Kit (Double Reagents)

- Catalogue Code: MAES0144
- Size: 100Assays
- Research Use Only

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

100Assays

Range:

0.2-12 mmol/L

Storage:

2-8°C for 12 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 12 months.

Do not use components from different batches of kit.

2. Background

Cholesterol is often present in the form of lipoproteins in the blood, and low-density lipoprotein in plasma is the main carrier for transporting endogenous cholesterol, which is degraded and converted by binding to low-density lipoprotein receptors on its cell membrane. LDL-C is the main lipoprotein in fasting plasma, accounting for about 2/3 of plasma lipoproteins, and is the main vehicle for transporting cholesterol to extrahepatic tissues. The defect of LDL-R function will lead to the decrease of the clearance ability of plasma LDL-C, and eventually lead to the formation of atherosclerotic plaque in the artery. Therefore, the content of LDL-C is related to the incidence of cardiovascular disease and the degree of lesions, and is considered to be the main pathogenic factor of atherosclerosis. Its concentration is significantly positively correlated with the incidence of coronary heart disease. It is also an evaluation of individual coronary heart disease. An important indicator of the risk factors that occur.

3. Intended Use

This kit can be used for detection of low-density lipoprotein cholesterol (LDL-C) content in serum, plasma, cells and tissue samples.

4. Detection Principle

Lipoproteins (except LDL) such as HDL, CM, and 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 are still intact, and then the reagent containing coupling agent is added, which can dissociate the LDL particles to release cholesterol, which is catalyzed by cholesterol esterase (CE) and cholesterol oxidase (CO) and produce hydrogen peroxide. Hydrogen peroxide is catalyzed by oxidase (POD) in the presence of 4-aminoantipyrine (4-AA) and phenol (T-OOS) to form a red quinone compound. The coloured substance have a maximum absorption peak at 546 nm. Measure the OD value at 546 nm and the LDL-C content in the sample can be calculated.

5. Kit Components & Storage

Item	Specification	Storage
Enzyme working Solution 1	75 mL × 1 vial	2-8°C, 12 months, avoid direct sunlight
Enzyme working Solution 2	25 mL × 1 vial	2-8°C, 12 months, avoid direct sunlight
Standard	Lyophilized × 1 vial	2-8°C, 12 months, avoid direct sunlight

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Spectrophotometer (546 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)

6. Assay Notes:

1. Prevent the formulation of bubbles when adding the liquid to the microplate.

2. Protect the reagent from contamination of glucose, cholesterol, etc.

7. Reagent Preparation:

1. Bring all reagents to room temperature before use.

2. **Preparation of standard solution:** Dissolve a vial of standard powder with double distilled water (refer to the delivery manual for volume) before use.

8. Sample Preparation

1. Serum sample:
Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) and preserve on ice before detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:
Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g 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) and preserve on ice before 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 (2×10^6): normal saline (0.9% NaCl) (μL) =1: 200. Sonicate the sample on an ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve on ice before 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. Use filter paper to absorb excess water and weigh. Homogenize at the ratio of the volume of normal saline (0.9% NaCl) (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 and preserve on ice before detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Note: If the tissue sample is high-fat sample or partly high lipid sample, the homogenate medium should be absolute alcohol.

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 (0.2-12 mmol/L).

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
Rat plasma	1
Porcine serum	1
10% Mouse kidney tissue homogenate	1
10% Rat liver tissue homogenate	1
HepG2 cells	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 546 nm

10. Operation Steps

Operation Table

	Blank tube	Standard tube	Sample tube
Double distilled water (μL)	10		
Standard (μL)		10	
Sample (μL)			10
Enzyme working Solution 1 (μL)	750	750	750
Mix fully and incubate at 37°C for 5 min. Set the spectrophotometer to zero with distilled water and measure the absorbance value (A_1) of each tube at 546 nm wavelength with 0.5 cm optical path cuvette.			
Enzyme working Solution 2 (μL)	250	250	250
Mix fully and incubate at 37°C for 5 min. Set the spectrophotometer to zero with double distilled water and measure the absorbance value (A_2) of each tube at 546 nm wavelength with 0.5 cm optical path cuvette.			

11. Calculations

1. Serum (Plasma) sample:

LDL-C content (*mmol/L*)

$$= \frac{(\text{Sample A2}-\text{SampleA1})-(\text{Blank A2}-\text{BlankA1})}{(\text{Standard A2}-\text{StandardA1})-(\text{BlankA2}-\text{BlankA1})} \times \text{Concentration of standard } (mmol/L)$$

2. Tissue sample:

When homogenate medium is phosphate buffer or normal saline, the formula is as follows:

$$\textbf{LDL}-\textbf{C content } \left(\frac{\boldsymbol{mmol}}{\boldsymbol{gprot}}\right) =$$

$$\frac{(\text{Sample A2} - \text{SampleA1}) - (\text{Blank A2} - \text{BlankA1})}{(\text{Standard A2} - \text{StandardA1}) - (\text{BlankA2} - \text{BlankA1})} \times \text{Concentration of standard } \left(\frac{mmol}{L}\right)$$

$$\div \text{ Protein concentration of tested sample } (gprot/L)$$

When homogenate medium is absolute alcohol, the formula is as follows:

LDL-C content (mmol/g tissue)

$$= \frac{(\text{Sample A2} - \text{SampleA1}) - (\text{Blank A2} - \text{BlankA1})}{(\text{Standard A2} - \text{StandardA1}) - (\text{BlankA2} - \text{BlankA1})}$$

$$\times \text{Concentration of standard (mmol/L)} \times \text{Volume of absolute alcohol (L)}$$

$$\div \text{ Weight of tested sample (g)}$$

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12. Performance Characteristics

Detection Range	0.2-12 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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