Technical Manual

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

- **Catalogue Code: MAES0143**
- **Size: 96T**
- **Research Use Only**

1. Key Features and Sample Types:

Detection method:

Colorimetric method

Specification:

96T

Range:

0.04-12 mmol/L

Sensitivity:

0.04 mmol/L

Storage:

2-8°C for 6 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 6 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	18 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Enzyme working Solution 2	6 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Standard	Lyophilized × 1 vial	2-8°C, 6 months, avoid direct sunlight
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (530-570 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 formation 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.

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 (0.04-12 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

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85
B	B	B	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86
C	S1	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
D	S2	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88
E	S3	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
F	S4	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
G	S5	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
H	S6	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92

Note: A, blank wells; B, standard wells; S1-S92, sample wells.

10. Operation Steps:

Operation table with 96 wells microplate reader

	Blank well	Standard well	Sample well
Double distilled water (μL)	2.5		
Standard solution (μL)		2.5	
Sample (μL)			2.5
Enzyme working Solution 1 (μL)	180	180	180
Mix fully and incubate at 37°C for 5 min. Measure the OD value (A_1) at 546 nm with microplate reader.			
Enzyme working Solution 2 (μL)	60	60	60
Mix fully and incubate at 37°C for 5 min. Measure the OD value (A_2) at 546 nm with microplate reader. $\Delta A=A_2-A_1$			

Operation table with automatic biochemical analyser

Setting parameter

Main wavelength	546 nm
Reaction type	Terminal method
Reaction direction	Up reaction (+)

Operation steps

Sample/ Double distilled water (μL)	2.5
Enzyme working Solution 1 (μL)	180
Mix fully and incubate at 37°C for 5 min. Measure the OD value (A1) at 546 nm with biochemical analyzer.	
Enzyme working Solution 2 (μL)	60
Mix fully and incubate at 37°C for 5 min. Measure the OD value (A_2) at 546 nm with biochemical analyzer.	

11. Calculations:

1. Serum (plasma) and other liquid sample:

Operate with microplate reader:

$$\text{LDL-C content } (mmol/L) = (\Delta A_{Sample} - \Delta A_{Blank}) \div (\Delta A_{Standard} - \Delta A_{Blank}) \times c \times f$$

Operate with automatic biochemical analyzer:

$$\text{LDL-C content } (mmol/L) = \Delta A_{Sample} - \Delta A_{Standard} \times c \times f$$

2. Tissue sample:

Operate with microplate reader:

$$\text{LDL-C content } (mmol/g\ fresh\ weight) = (\Delta A_{Sample} - \Delta A_{Blank}) \div (\Delta A_{Standard} - \Delta A_{Blank}) \times c \times f \times V \div W$$

Operate with automatic biochemical analyzer:

$$\text{LDL-C content } (mmol/g\ fresh\ weight) = \Delta A_{Sample} - \Delta A_{Standard} \times c \times f \times V \div W$$

3. Cell sample:

Operate with microplate reader:

$$\text{LDL-C content } (mmol/10^6 g) = (\Delta A_{Sample} - \Delta A_{Blank}) \div (\Delta A_{Standard} - \Delta A_{Blank}) \times c \times f \times V \div N$$

Operate with automatic biochemical analyzer:

$$\text{LDL-C content } (mmol/10^6) = \Delta A_{Sample} \div \Delta A_{Standard} \times c \times f \times V \div N$$

ΔA: $A_2 - A_1$

c: The concentration of standard

f: Dilution factor of sample before tested

w: The weight of tissue sample, g

V: The volume of homogenization medium, L

N: The number of cells. For example, the number of cells is $5*10^6$, N is 5

12. Performance Characteristics:

Detection Range	0.04-12 mmol/L
Sensitivity	0.04 mmol/L
Average inter-assay CV (%)	10
Average intra-assay CV (%)	5.5

Analysis

Take 2.5 μL of mouse serum, carry the assay according to the operation table.

The results are as follows:

The average OD value of the blank (A_1) is 0.043, the average OD value of the blank (A_2) is 0.071, the average OD value of the standard (A_1) is 0.061, the average OD value of the standard (A_2) is 0.394, the average OD value of the sample (A_1) is 0.052, the average OD value of the sample (A_2) is 0.120, and the calculation result is:

$$\text{LDL-C } (mmol/L) = \frac{(0.120-0.052)-(0.071-0.043)}{(0.394-0.061)-(0.071-0.043)} \times 2.75\ mmol/L$$

$$= 0.36\ mmol/L$$

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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