PRODUCT INFORMATION & MANUAL

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

NBP3-25881

For research use only.
Not for diagnostic or therapeutic procedures.

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

Catalog No: NBP3-25881

Method: Colorimetric method

Specification: 96T (Can detect 92 samples without duplication)

Instrument: Microplate reader, biochemical analyzer

Sensitivity: 0.04 mmol/L

Detection range: 0.04-12 mmol/L

Average intra-assay CV (%): 5.5

Average inter-assay CV (%): 10

▲ This kit is for research use only.
▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

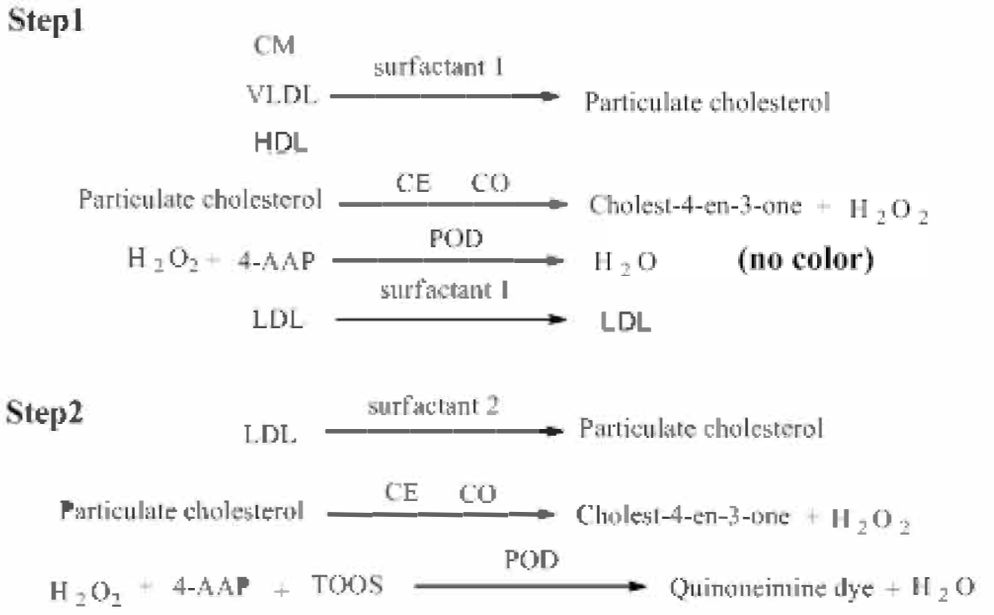
This kit can be used for detection of low-density lipoprotein cholesterol (LDL-C) content in serum, plasma, cells, culture supernatant and tissue samples.

▲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.

▲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.



▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Enzyme Working Solution 1	18 mL × 1 vial	2-8℃，12 months shading light
Reagent 2	Enzyme Working Solution 2	6 mL × 1 vial	2-8℃，12 months shading light
Reagent 3	Standard (Refer to the label of reagent 3 for concentration)	Powder × 1 vial	2-8℃，12 months shading light
Microplate		96 wells	No requirement
Plate Sealer		2 pieces	
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.			

▲Materials prepared by users

Instruments

Microplate reader (546 nm) or Biochemical analyzer (546 nm), Micropipettor, Water bath, Incubator, Vortex mixer, Centrifuge

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL, 5 mL)

Reagents

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

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

1. Prevent the formulation of bubbles when adding the liquid to the microplate.
2. Protect the reagent from contamination of glucose, cholesterol, etc.
3. The amount of reagent and sample can be increased and decreased proportionately according to the volume of cuvette.

Pre-assay preparation

▲Reagent preparation

1. Bring all reagents to room temperature before use.
2. The preparation of standard solution:
   Dissolve a vial of standard powder with 200 μL double distilled water before use.The prepared solution can be stored at 2-8℃ for 2 weeks with shading light.

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and 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 of serum (plasma) is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4); The diluent of animal tissue or cells is isopropanol.

Assay protocol

▲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.

▲Operation table

1. Operate with microplate reader

	Blank well	Standard well	Sample well
Double distilled water (μL)	5		
Standard (μL)		5	
Sample (μL)			5
Reagent 1 (μL)	180	180	180
Mix fully and incubate at 37℃ for 5 min. Measure the OD value (A1) at 546 nm with microplate reader.			
Reagent 2 (μL)	60	60	60
Mix fully and incubate at 37℃ for 5 min. Measure the OD value (A2) at 546 nm with microplate reader. ΔA=A2-A1.			

2. Operate with automatic biochemical analyzer

a. Setting parameter

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

b. Operation steps

Sample/ Double distilled water (μL)	5
Reagent 1 (μL)	180
Mix fully and incubate at 37℃ for 5 min. Measure the OD value (A1) at 546 nm with biochemical analyzer.	
Reagent 2 (μL)	60
Mix fully and incubate at 37℃ for 5 min. Measure the OD value (A2) at 546 nm with biochemical analyzer. ΔA=A2-A1.	

▲Calculation

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

a. Operate with microplate reader:

$$\text{LDL-C (mmol/L)} = \frac{\Delta A_{sample} - \Delta A_{blank}}{\Delta A_{standard} - \Delta A_{blank}} \times c \times f$$

b. Operate with automatic biochemical analyzer:

$$\text{LDL-C (mmol/L)} = \frac{\Delta A_{sample}}{\Delta A_{standard}} \times c \times f$$

2. Tissue samples:

a. Operate with microplate reader:

$$\text{LDL-C (mmol/g fresh weight)} = \frac{\Delta A_{sample} - \Delta A_{blank}}{\Delta A_{standard} - \Delta A_{blank}} \times c \times f \times V \div W$$

b. Operate with automatic biochemical analyzer:

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

3. Cells samples:

a. Operate with microplate reader:

$$\text{LDL-C (mmol/}10^6\text{)} = \frac{\Delta A_{sample} - \Delta A_{blank}}{\Delta A_{standard} - \Delta A_{blank}} \times c \times f \times V \div N$$

b. Operate with automatic biochemical analyzer:

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

Note:

c: Concentration of standard.

f: Dilution factor of sample before test.

V: Volume of isopropanol (L).

W: Weight of sample (g).

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

Appendix I Data

▲Example analysis

Take 5 μL of mouse serum sample and carry the assay with microplate reader according to the operation table. The results are as follows:

The average OD value of the blank (A1) is 0.043, the average OD value of the blank (A2) is 0.071, the average OD value of the standard (A1) is 0.061, the average OD value of the standard (A2) is 0.394, the average OD value of the sample (A1) is 0.052, the average OD value of the sample (A2) is 0.120, and the calculation result is:

$$\text{LDL-C (mmol/L)} = \frac{(0.120-0.052)-(0.071-0.043)}{(0.394-0.061)-(0.071-0.043)} \times 2.75 = 0.36 \text{ mmol/L}$$

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection.

▲Plasma

Take fresh blood into the tube which has anticoagulant (Heparin is used as anticoagulant), centrifuge at 700-1000 g for 10 min at 4℃ . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection.

▲Cell culture supernatant

Collect the culture supernatant, centrifuge at 1000 g for 10 min, and take the supernatant for detection.

▲Tissue

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8℃ . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4℃ . Take the supernatant to preserve it on ice for detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for a month.

▲Cells

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 (10^6): homogenization medium (μL) =1: 300-500. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80℃ for a month.

Note:

1. Homogenized medium: Isopropanol

2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.
Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

(3) Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2 s/ time, interval for 3 s, the total time is 5 min).

Appendix III References

1. Brown M S, Goldstein J L. Receptor-mediated control of cholesterol metabolism[J]. Science, 1976, 191(4223): 150-154.
2. Elsulaiman A A. Sensitivity analysis of the machine's controller and the impact of machine data on over-all stability studies[J]. 1977: 226-241.