Technical Manual

High-density Lipoprotein Cholesterol (HDL-C) Colorimetric Assay Kit (Double reagents)

- Catalogue Code: MAES0149
- Size: 100 Assays
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

100 Assays

Range:

0.065-3.8 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

High-density lipoprotein cholesterol is mainly synthesized in the liver. It is an anti-atherosclerotic lipoprotein that can transport cholesterol from extrahepatic tissues to the liver for metabolism and excretion of bile from the body. Its plasma level is negatively correlated with the risk of cardiovascular disease. High-density lipoprotein can take cholesterol from the cell membrane, catalyzed by lecithin cholesterol acyltransferase to form cholesterol ester, and then transfer the carried cholesterol ester to very low density lipoprotein and low density lipoprotein. High-density lipoprotein cholesterol content is relatively fixed, containing about 20% to 30% of the total body cholesterol.

3. Intended Use

This kit can be used for detection of high-density lipoprotein cholesterol (HDL-C) content in serum, plasma samples.

4. Detection Principle

CM, VLDL and LDL coagulate in a polyanionic environment to form a polymer and are masked by the polymer. High-density lipoprotein (HDL) form soluble compounds under the action of a surfactant, so that HDL-C can directly react with enzyme reagents containing cholesterol esterase (CE) and cholesterol oxidase (CO) to 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 HDL-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, 12 months, shading light
Enzyme working Solution 2	6 mL × 1 vial	2-8°C, 12 months, shading light
Standard	Powder × 1 vial	2-8°C, 12 months, shading light

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:

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

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

2. Plasma sample:
Take fresh blood into the tube which has anticoagulant (Heparin is used as anticoagulant and it is 10-12.5 IU of Heparin into 1 mL blood), 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.

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 (0.065-3.8 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
Mouse plasma	1
Rat serum	1
Rat plasma	1
Porcine serum	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 double distilled water and measure the OD value (A_1) of each tube at 546 nm with 0.5 cm optical path cuvette.			
Enzyme working Solution 2 (μL)	250	250	250
Mix fully and incubate at 37℃ for 5 min. Set the spectrophotometer to zero with double distilled water and measure the OD value (A_2) of each tube at 546 nm with 0.5 cm optical path cuvette, $\triangle A=A_2-A_1$.			

11. Calculations

HDL-C content (*mmol/L*)

$= (\Delta A_{Sample} - \Delta A_{Blank}) \div (\Delta A_{Standard} - \Delta A_{Blank}) \times c \times f$

c: The concentration of standard

f: Dilution factor of sample before test

12. Performance Characteristics

Detection Range	0.065-3.8 mmol/L

Email: info@assaygenie.com **Web:** www.assaygenie.com

Safety Notes

Some of the reagents in the kit contain dangerous substances. Avoid 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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