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

Catalog Number EEA012 (96 tests)

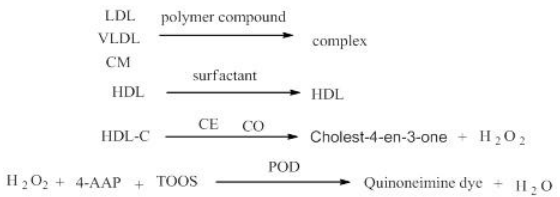
Rev 3.0

For safety and biohazard guidelines, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

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

CM, VLDL and LDL coagulate in a polyanionic environment 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.



Contents and storage

Kit and components are shipped at 2-8℃. An unopened kit can be stored at 2-8℃ for 12 months.

Components	Quantity (96 tests)
Enzyme Working Solution 1	18 mL
Enzyme Working Solution 2	6 mL
Standard (Refer to the label for concentration)	Powder
Microplate	1 plate
Plate Sealer	2 pieces

Required materials

- Distilled or deionized water
- Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)
- Microtiter plate reader with software capable of measurement at or near 546 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution
- Incubator capable of maintaining 37˚C.

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Sample preparation guidelines

Serum and plasma samples: Detect the sample directly. If the sample is turbid, centrifuge at 10000 g for 10 min at 4℃, then take the supernatant for detection.

Prepare samples

It is recommended to take 2~3 samples with expected large difference to do a pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.06-3.8 mmol/L).

Note: Use all samples within 2 hours of dilution

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).

Prepare enzyme working solution 1 and enzyme working solution 2

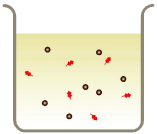
Incubate enzyme working solution 1 and enzyme working solution 2 at 25℃ for 15 min with the amount required for the experiment, and the remaining reagents were stored at 2-8℃.

Prepare standard solution

Note: Use glass or plastic tubes for diluting standards.

- Dissolve a vial of standard powder with 200 μL distilled or deionized water before use. The prepared solution can be stored at 2-8℃ for 2 weeks with shading light.

1. Assay procedure for 96-well microplate reader

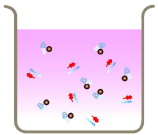


1. Add sample, standard and blank

a. Standard well: add 5 μL of standard solution.

b. Sample well: add 5 μL of sample.

c. Blank well: add 5 μL of distilled water.



2. Add substrate

a. Add 180 μL enzyme working solution 1 into each well.

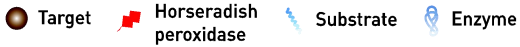
b. Mix fully and incubate at 37°C for 5 min.

c. Measure the OD value (A_1) of each well with microplate reader at 546 nm.

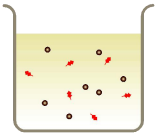
d. Add 60 μL enzyme working solution 2 into each well.

e. Mix fully and incubate at 37°C for 5 min.

f. Measure the OD values (A_2) of each well with microplate reader at 546 nm



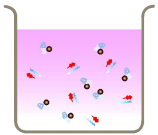
2. Assay procedure for Automatic biochemical analyzer



1. Add sample and blank

a. Sample well: add 5 μL of sample.

b. Blank well: add 5 μL of distilled water.



2. Add substrate

c. Add 180 μL enzyme working solution 1 to each well.

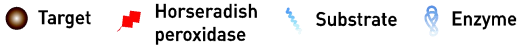
d. Mix fully and incubate at 37°C for 5 min.

e. Measure the OD value (A_1) at 546 nm with biochemical analyzer.

f. Add 60 μL enzyme working solution 2 to each well.

g. Mix fully and incubate at 37°C for 5 min.

h. Measure the OD value (A_2) at 546 nm with biochemical analyzer. $\Delta A=A_2-A_1$.



Calculation

Serum (plasma) and other liquid sample:

Operated with microplate reader:

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

Operated with automatic biochemical analyzer:

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

[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.

To easy calculate the test results, refer to the calculation file available on the webpage.

Example analysis

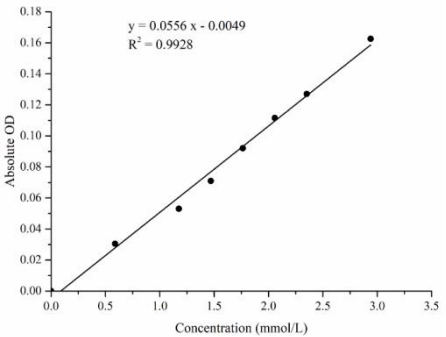
Take 5 μL of mouse serum sample and carry the assay with microplate reader according to the operation steps. The results are as follows: the average OD value of the blank (A_1) is 0.043, the average OD value of the blank (A_2) is 0.059, the average OD value of the standard (A_1) is 0.064, the average OD value of the standard (A_2) is 0.172, the average OD value of the sample (A_1) is 0.050, the average OD value of the sample (A_2) is 0.246, and the calculation result is:

$$\underset{(\text{mmol/L})}{\text{HDL-C}} = \frac{(0.246 - 0.050) - (0.059 - 0.043)}{(0.172 - 0.064) - (0.059 - 0.043)} \times 1.1 \text{ mmol/L} = 2.15 \text{ mmol/L}$$

Performance characteristics

- Standard curve (example)

The following data were obtained for the various standards over the range of 0-2.94 mmol/L standard.

Concentration (mmol/L)	OD at 546 nm	Standard Curve
2.94	0.164	
2.35	0.128	
2.06	0.113	
1.76	0.093	
1.47	0.072	
1.18	0.054	
0.59	0.032	
0.00	0.001	

- Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	1.29	2.08	2.97
%CV	4.9	5.2	5.1

CV = Coefficient of Variation

- Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	1.36	2.04	2.82
%CV	3.0	2.9	3.2

CV = Coefficient of Variation

- Expected values

This assay was tested with human serum, and plasma samples without dilutions.

Sample Type	Range (**mmol/L**)	Average (**mmol/L**)
Serum	1.502–1.653	1.604
Plasma (EDTA and heparin)	1.026–1.212	1.164

- Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 97%.

	Sample 1 (low conc.)	Sample 2 (middle conc.)	Sample 3 (high conc.)
Expected Conc. (mmol/L)	0.5	1.5	2
Observed Conc. (mmol/L)	0.48	1.42	1.98
Recovery rate (%)	96	95	99

- Recommended 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.												

- Sensitivity

The analytical sensitivity of the assay is 0.06 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.

Limited product warranty

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