FUJIFILM

HDL-C L-Type

Read this instruction sheet thoroughly before use!

Stable liquid Reagent - Immunoinhibition Method

For the quantitative determination of HDL-Cholesterol in Serum

CE

412-72395
0124D3

Intended use

The HDL-C L-Type test is an *in vitro* assay for the quantitative determination of high density lipoprotein cholesterol (HDL-C) in serum.

Summary and explanation of the test

Lipoprotein is classified into low density lipoprotein (LDL), very low density lipoprotein (VLDL), and chylomicrons (CM) according to specific gravity. In recent years, in addition to total cholesterol, high density lipoprotein cholesterol (HDL-C) has become an important tool used to assess an individual risk of developing coronary heart disease (CHD) since a strong negative relationship between HDL-C concentration and CHD was reported[1,2].

HDL-C had been assayed by ultracentrifugation, gel filtration, electrophoresis, high performance liquid chromatography (HPLC), precipitation, and direct process[3]. Recently, direct process has been widely used. HDL-C is a liquid type reagent that assays HDL-cholesterol in serum directly by employing antibody.

Principle of the method

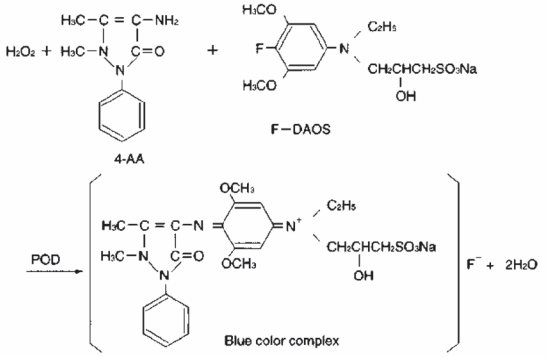
Anti human β - lipoprotein antibody in R1 binds to lipoproteins (LDL, VLDL, and chylomicrons) other than HDL. The antigen-antibody complexes formed block enzyme reactions when R 2 is added. Cholesterol esterase (CHE) and cholesterol oxidase (CO) in R 2 react only with HDL-C. Hydrogen peroxide produced by the enzyme reactions with HDL-C yields a blue color complex upon oxidative condensation of F-DAOS [N-ethyl-N-(2-hydroxy-3-sulfopropyl)--3,5-dimethoxy- 4-fluoroaniline, sodium salt] and 4-aminoantipyrine (4-AA) in the presence of peroxidase (POD).

By measuring the absorbance of the blue color complex produced, the HDL-C concentration in the sample can be calculated when compared with the absorbance of the HDL-C Calibrator.

Reactions

LDL, VLDL and chylomicrons —(Anti human β- lipoprotein antibody)→ Antigen-Antibody complex

HDL-Cholesterol + H_2O + O_2 —(CHE & CO)→ Δ^4 - Cholestenone + Fatty acid + H_2O_2



Reagents

Contents and storage conditions

R1:	Pretreatment	Store at 2-10°C*
R2:	Enzyme Reagent	Store at 2-10°C* (*Do not freeze)

Ingredients

R1: Pretreatment	Good's Buffer (pH 7.0)	30 mmol/L
	4-AA	0.9 mmol/L
	POD	2.4 IU/mL
	Ascorbate Oxidase	2.7 IU/mL
	Anti human β–lipoprotein antibody	
	Mixture containing: 5-Chloro-2-methyl-2H-isotiazol-3-one [EG No 247-500-7] and 2-Methyl-2H-isothiazol-3-one [EG No 220-239-6] (3:1) 0.0015-0.06%	
R2: Enzyme Reagent	Good's Buffer (pH 7.0)	30 mmol/L
	CHE	4.0 IU/mL
	CO	20 IU/mL
	F-DAOS	0.8 mmol/L

Reagent preparation

R1: Use as supplied.
After opening the bottle, this solution is stable for 30 days at 2-10°C.

R2: Use as supplied.
After opening the bottle, this solution is stable for 30 days at 2-10°C.

Samples

Serum can be used as a specimen.
It is recommended to measure HDL-C immediately after collection.

Physical or chemical indications of instability

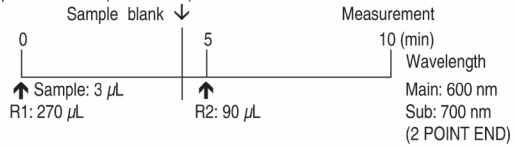
The presence of precipitates in the reagents or values of control sera outside the manufacturer's acceptable range may be an indication of reagent's instability.

Instruments

The reagent is designed to be used on commercially available automated analyzers. Refer to the operating manual for a description of instrument operation and specifications.
A validation by the user in practice at the customer's site in the form of measurements of adequate control or patient sera in sufficient number is indispensable.

Standard procedure

Temperature: 37°C (Hitachi®717)



Calibrator: Wako HDL-C Calibrator (Available separately.)

Calculation of HDL-C concentration

Calculate HDL-C concentration from the calibration curve which was created from absorbance of calibrator.

Application to the various automatic analyzers

Input the parameters according to the instructions of instruments to perform the measurement. Instrument applications are available upon request.

Results

The final results are automatically calculated and printed in concentration. The results are given in mg/dL.

Expected values[4]

Low HDL-Cholesterol	< 40 mg/dL
High HDL-Cholesterol	> 60 mg/dL

Performance characteristics

(1) **Accuracy**
When a sample of known concentration is assayed, the assay value falls within the range of ± 10% of the known concentration.

(2) **Sensitivity**
a) When saline is assayed, the absorbance is not more than 0.1.
b) When a calibrator of given concentration (50 mg/dL) is assayed, the absorbance is 0.07 – 0.34.

(3) **Precision**
When a sample is assayed 5 times in a run, CV is not more than 5%.

(4) **Measurable range**
0.9-180 mg/dL HDL-cholesterol (In the case of using the standard procedure).

Correlation

Specimen	Serum
Correlation coefficient	r = 0.998 (n = 50)
Regression equation	y = 0.96 x + 2.5
y	Wako HDL-C L-Type (mg/dL)
x	Product of company A (mg/dL)

Interfering substances

Hemolysis, ascorbic acid and bilirubin do not have significant effects on the assay.

Manufacturer:
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Wako

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Warnings and precautions

- For *in vitro* diagnostic use.
- The usage and application of this test is reserved for professional use only. Please refer to respective national and local regulations and legislation.
- Not to be used internally in humans or animals.
- Do not use the reagents described above for any purpose other than described herein. Performance cannot be guaranteed if the reagents are used in other procedures or for other purposes.
- Operate the instruments according to operator's manuals under appropriate conditions.
- When using enzymatic methods for the determination of cholesterol esters, contamination and interference to other clinical chemistry assays on the same instrument in principle cannot be excluded. In the rare event of such a problem occurring, please refer to the instrument's manual for channel setting and washing procedure options.
- Clinical diagnosis must be determined with clinical symptoms and other test results by a physician.
- Store the reagents under the specified conditions. Do not use reagents past the expiration date stated on each reagent container label.
- Do not use reagents which were frozen in error. Such reagents may give false results.
- After opening the reagents, it is recommended to use them immediately. When the opened reagents are stored, cap the bottles and keep them under the specified conditions.
- Do not use the containers and other materials in the package for any purposes other than those described herein.
- When triglyceride in a sample exceeds 1,200 mg/dL, dilute the sample with a saline solution, repeat assay and multiply result by the dilution factor.
- Since all specimens are potentially infectious, they should be handled with appropriate precaution. Refer to respective good laboratory practice protocols for preventing transmission of infection and handle samples in accordance with any other local or national regulations relating to the safe handling of such materials.
- If the reagents come in contact with the mouth, eyes or skin, wash off immediately with a large amount of water. Consult a physician if necessary.
- When discarding the reagents, dispose of them according to local or national regulations.

Additional information (R1, R2)

EUH208 Contains Mixture of 5-Chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one. May produce an allergic reaction.

Quality control

A quality control program is recommended for all clinical laboratories.

References

1. Rifai, N. e Warnick, G.R., Hsg. Laboratory Measurement of Lipids, Lipoproteins and Apolipoproteins AACC Press, Washington, DC, USA, 1997.
2. Recommendation of the Second Joint Task Force of European and other Societies on Coronary Prevention. Prevention of coronary heart disease in clinical practice; Eur Heart J 1998; 19 : 1434 - 503.
3. Gordon, T., Castelli, W.P., Hjortland, M. C., et al., Am. J. Med. 62 : 707 - 714 (1977).
4. Lipid Liga e.V. in accordance with the third report of the National Cholesterol Education Program (NCEP).

Ordering information

Code No.	Products	Package
412-72395	HDL-C L-Type R1	R1: 4 x 60 mL
412-72495	HDL-C L-Type R2	R2: 4 x 20 mL
418-72395	HDL-C L-Type R1	R1: 4 x 270 mL
418-72495	HDL-C L-Type R2	R2: 4 x 90 mL
416-51095	HDL-C Calibrator	CAL: 4 x for 3 ml

Manufacturer:

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