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# HDL-CHOLESTEROL

STABLE LIQUID

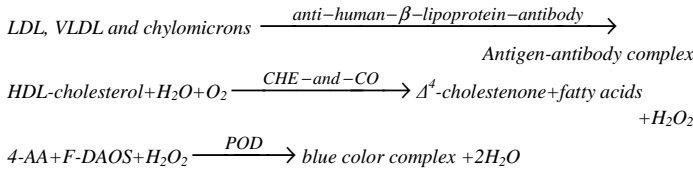


Cat. No.:	47661	47662
	60 ml	500 ml
	(1x45 ml+1x15 ml)	(1x375 ml +1x125 ml)

## A direct immunoinhibition method for the quantitative determination of high density lipoprotein cholesterol (HDL-C) in serum.

### Principle

Anti human  $\beta$ -lipoprotein antibody in Reagent 1 binds to all lipoproteins of the serum (LDL, VLDL, and chylomicrons) other than HDL. The antigen-antibody complexes formed block enzyme reactions started when Reagent 2 is added. Cholesterol esterase (CHE) and cholesterol oxidase (CO) in Reagent 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 N-ethyl-N-(2-hydroxy-3-sulfo-propyl)-3,5-dimethoxy-4-fluoroaniline, sodium salt (F-DAOS) and 4-aminoantipyrine (4AA) in the presence of peroxidase (POD). By measuring absorbance of the blue color complex produced, at the near optimum wavelength of 593 nm, the HDL-C concentration in the sample can be calculated when compared with the absorbance of the HDL-C Calibrator.



### Reference values

**Adult male:** 0.92-2.07 mmol/l (35.3-79.5 mg/dl)

**Adult female:** 1.09-2.29 mmol/l (42.0-88.0 mg/dl)

It is recommended that each laboratory should assign its own normal range.

### Reagents

1. Pretreatment solution: Store at 2-10°C. Do not freeze. 30 mmol/l Goods buffer, (pH=7.0), containing 4-AA (0.9 mmol/l), POD (2400 U/l), ascorbate oxidase (2700 U/l), and anti human  $\beta$ -lipoprotein antibody.

2. Enzyme reagent: Store at 2-10°C. Do not freeze. 30 mmol/l Goods buffer, (pH=7.0), containing CHE (4000 U/l), CO (20000 U/l), and F-DAOS (0.8 mmol/l).

### Samples

Use serum as a specimen. It is recommended to measure HDL-C immediately after collection. Ascorbic acid, bilirubin, and hemoglobin do not have a significant effect on the measurement.

### PROCEDURE

#### Working reagent

Reagents 1 and 2: are ready for use.

#### Assay conditions

Main wavelength: 600 nm

Sub wavelength: 700 nm

Light path: 1 cm

Temperature: 37 °C

Method: endpoint (increasing)

#### Pipette into cuvette

	Reagent blank	Standard	Sample
Reagent R1	270 $\mu$ l	270 $\mu$ l	270 $\mu$ l
Standard		3 $\mu$ l	
Sample			3 $\mu$ l
Distilled water	3 $\mu$ l		

Mix and incubate for 5 minutes.

	Reagent R2	90 $\mu$ l	90 $\mu$ l	90 $\mu$ l

Mix and incubate for 5 minutes, then read the final absorbance value against reagent blank.

#### Calibration

S1: Distilled water

S2: Sentinel HC calibrator

Wako HC calibrator

Randox HC calibrator

#### Calibration frequency

Two point calibration is recommended:

- after reagent lot change,

- as required following quality control procedures.

### Calculation

$$\frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times C_{\text{Standard}} = C_{\text{Sample}}$$

A = absorbance

C = concentration

### Quality control

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits

### PERFORMANCES DATA

#### Linearity

The test is linear up to 7,15 mmol/l (275 mg/dl).

The reagent is linear between the concentration of 1-180 mg/dl (0.026-4.69 mmol/l). When the concentration of triglyceride in a sample exceeds 1200mg/dl (13.4 mmol/l), dilute the sample with a saline solution, repeat assay and multiply result by the dilution factor.

#### Sensitivity

Absorbance of sample blank is 0.1 or less. Absorbance of a 50 mg/dl (1,30 mmol/l) HDL-cholesterol sample is between 0.07 and 0.34.

#### Specificity

Obtained values of control serum samples with known amount of HDL-cholesterol fall within +/- 10%.

#### Precision

	Repeatability
	CV%
Serum	<5

#### Correlation

A comparative study has been performed between our reagent and other commercial HDL-cholesterol reagent on 50 human serum samples.

The parameters of linear regression are as follows:

	Serum
n=	50
Regression analysis	y= 0.96x+ 0.065 mmol/l
Correlation coefficient	r=0.998

#### Note

Do not use the pretreatment reagent, which was frozen by mistake.

#### For in vitro diagnostic use only.

#### The following symbols are used on labels

For in vitro diagnostic use

Use by (last day of the month)

Temperature limitation

Batch Code

Code

#### Bibliography

Rifai, N., Warnick, GR. (eds.) *Laboratory Measurement of Lipids, Lipoproteins and Apolipoproteins*. AACC Press, Washington, D.C., USA, 1994.

2. Burtis, CA., Ashwood, E.R. (eds.) *Tietz Textbook of Clinical Chemistry*. 2<sup>nd</sup> ed. Saunders, Philadelphia, 1994.

3. Gordon, T., Castelli, W.P., Hjortland, M.C., et al., *Am. J. Med.* 62,707-714 (1977)