HDL-C Immuno FS*

Diagnostic reagent for quantitative in vitro determination of high density lipoprotein cholesterol (HDL-C) in serum or plasma on photometric systems

Order Information

Cat. No.	Kit size						
1 3521 99 10 021	R1	5 x	20 mL	+	R2	1 x	25 mL
1 3521 99 10 026	R1	5 x	80 mL	+	R2	1 x	100 mL
1 3521 99 10 023	R1	1 x	800 mL	+	R2	1 x	200 mL
1 3521 99 10 704	R1	8 x	50 mL	+	R2	8 x	12.5 mL
1 3521 99 10 917	R1	8 x	60 mL	+	R2	8 x	15 mL
1 3521 99 10 930	R1	4 x	20 mL	+	R2	2 x	10 mL

Summary [1,2]

Cholesterol is a component of cell membranes and a precursor for steroid hormones and bile acids synthesized by body cells and absorbed with food. Cholesterol is transported in plasma via lipoproteins, namely complexes between lipids and apolipoproteins. There are four classes of lipoproteins: high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL) and chylomicrons. While LDL is involved in the cholesterol transport to the peripheral cells, HDL is responsible for the cholesterol uptake from the cells. The four different lipoprotein classes show distinct relationship to coronary atherosclerosis. LDL-cholesterol contributes to atherosclerotic plaque formation within the arterial intima and is strongly associated with coronary heart disease (CHD) and related mortality. Even with total cholesterol within the normal range an increased concentration of LDL-cholesterol indicates high risk. HDL-cholesterol has a protective effect impeding plaque formation and shows an inverse relationship to CHD prevalence. In fact, low HDL-cholesterol values constitute an independent risk factor. The determination of the individual total cholesterol (TC) level is used for screening purposes while for a better risk assessment it is necessary to measure additionally HDL-cholesterol and LDL-cholesterol.

In the last few years several controlled clinical trials using diet, life style changes and/or different drugs (especially HMG CoA reductase inhibitors [statins]) have demonstrated that lowering total cholesterol and LDL-cholesterol levels reduce the CHD risk drastically.

Method

Previous HDL-cholesterol determinations were performed by time consuming precipitation methods [3].

HDL-C Immuno FS is a homogeneous method for HDL-cholesterol measurement without centrifugation steps. Antibodies against human lipoproteins are used to form antigen-antibody complexes with LDL, VLDL and chylomicrons in a way that only HDL-cholesterol is selectively determined by an enzymatic cholesterol measurement [4].

Principle

$$\text{LDL, VLDL, Chylomicrons} \xrightarrow{\text{Anti-human } \beta\text{-lipoprotein antibodies}} \text{Antigen-antibody complexes} + \text{HDL}$$

$$\text{HDL-cholesterol} + H_2O + O_2 \xrightarrow{\text{CHE \& CHO}} \text{Cholest-4-en-3-one} + \text{fatty acid} + H_2O_2$$

$$H_2O_2 + \text{F-DAOS} + \text{4-Aminoantipyrine} \xrightarrow{\text{POD}} \text{Blue complex} + H_2O$$

Reagents

Components and Concentrations

R1:			
	Good's buffer	pH 7.0	25 mmol/L
	4-Aminoantipyrine		0.75 mmol/L
	Peroxidase	(POD)	2000 U/L
	Ascorbate oxidase		2250 U/L
	Anti-human β-lipoprotein antibody (sheep)		
R2:	Good's buffer	pH 7.0	30 mmol/L
	Cholesterol esterase	(CHE)	4000 U/L
	Cholesterol oxidase	(CHO)	20000 U/L
	N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxy-4-fluoroaniline, sodium salt (F-DAOS)		0.8 mmol/L

Storage Instructions and Reagent Stability

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C protected from light and contamination is avoided. Do not freeze the reagents!

Note: It has to be mentioned, that the measurement is not influenced by occasionally occurring color changes, as long as the absorbance of the premixed reagent (4 parts R1 + 1 part R2) is < 0.03 at 600 – 700 nm.

On board stability: 4 weeks at 2 – 8°C

Warnings and Precautions

1. Reagent 1: Warning. H317 May cause an allergic skin reaction. P280 Wear protective gloves/protective clothing/eye protection/face protection. P302+P352 If on skin: Wash with plenty of water/soap. P333+P313 If skin irritation or rash occurs: Get medical advice/attention.
2. The reagents contain animal material. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practices.
3. In very rare cases, samples of patients with gammopathy might give falsified results [8].
4. N-acetylcysteine (NAC), acetaminophen and metamizole medication leads to falsely low results in patient samples.
5. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
6. For professional use only!

Waste Management

Please refer to local legal requirements.

Reagent Preparation

The reagents are ready to use.

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Specimen

Serum, heparin plasma [5]

Stability:			
	2 days	at	20 – 25°C
	7 days	at	4 – 8°C
	3 months	at	−20°C

Discard contaminated specimens. Freeze only once.

Assay Procedure for Analyzers

Application sheets for automated systems are available on request.

Wavelength	600/700 nm (bichromatic measurement)
Optical path	1 cm
Temperature	37°C
Measurement	Against reagent blank

	Blank	Sample or calibrator
Sample or calibrator	-	2.4 µL
Reagent 1	240 µL	240 µL
Mix, incubate 5 min. at 37°C, read absorbance A1, then add:		
Reagent 2	60 µL	60 µL
Mix, incubate for 5 min. at 37°C, read absorbance A2.		

ΔA = (A2 – A1) sample or calibrator

Calculation

With calibrator

$$\text{HDL - C } [mg/dL] = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Calibrator}} \times \text{Conc. Calib.} [mg/dL]$$

Conversion factor

HDL-C [mg/dL] x 0.02586= HDL-C [mmol/L]

Calibrators and Controls

For the calibration of automated photometric systems, DiaSys' TruCal Lipid calibrator has to be used. The assigned values of the calibrator have been made traceable to NIST SRM® 1951 Level 2. DiaSys TruLab L control should be assayed for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
TruCal Lipid	1 3570 99 10 045	3 x 2 mL
TruLab L Level 1	5 9020 99 10 065	3 x 3 mL
TruLab L Level 2	5 9030 99 10 065	3 x 3 mL

Performance Characteristics

Measuring range

The test has been developed to determine HDL-C concentrations within a measuring range from 1 – 180 mg/dL (0.03 – 4.7 mmol/L). When values exceed this range the samples should be diluted 1 + 2 with NaCl solution (9 g/L) and the result multiplied by 3.

Specificity/Interferences

No interference was observed by ascorbic acid up to 50 mg/dL, hemoglobin up to 500 mg/dL, bilirubin up to 40 mg/dL and lipemia up to 1200 mg/dL triglycerides. For further information on interfering substances refer to Young DS [6].

Sensitivity/Limit of Detection

The lower limit of detection is 1 mg/dL (0.03 mmol/L).

Precision (n = 20)

Intra-assay precision	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	20.4	0.17	0.81
Sample 2	56.0	0.41	0.73
Sample 3	125	1.03	0.82

Inter-assay precision	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	44.0	0.83	1.88

Method Comparison

A comparison of DiaSys HDL-C Immuno FS (y) with a commercially available test (x) using 100 samples gave following results:

Y = 1.05 x + 0.571 mg/dL; r = 0.995

Reference Range [7]

National Cholesterol Education Program (NCEP) guidelines:

Low HDL-cholesterol (major risk factor for CHD):
< 40 mg/dL (< 1.04 mmol/L)

High HDL-cholesterol ("negative" risk factor for CHD):
≥ 60 mg/dL (≥ 1.55 mmol/L)

A number of factors contribute to low HDL-cholesterol levels: e.g. overweight and obesity, smoking, physical inactivity, drugs such as beta-blockers and progestational agents, genetic factors.

Each laboratory should check if reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature

1. Rifai N, Bachorik PS, Albers JJ. Lipids, lipoproteins and apolipoproteins. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 809-61.
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3. Wiebe DA, Warnick GR. Measurement of high-density lipoprotein cholesterol. In: Rifai N, Warnick GR, Dominiczak MH, eds. Handbook of lipoprotein testing. Washington: AACC Press, 1997. p. 127-44.
4. Nauck M, Maerz W, Wieland H. New immunoseparation-based homogenous assay for HDL-cholesterol compared with three homogenous and two heterogeneous methods for HDL-cholesterol. Clin Chem 1998; 44: 1443-51.
5. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001; p. 22-3.
6. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
7. Third Report of the National Cholesterol Education Program (NCEP). Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). NIH Publication No. 02-5215; September 2002.
8. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007;45(9):1240-1243.

Manufacturer

IVD CE

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