HDL-c direct FS*

Order Information

Cat. No.	Kit size		
1 3561 99 10 021	R1 5 x 20 mL	+	R2 1 x 25 mL
1 3561 99 10 026	R1 5 x 80 mL	+	R2 1 x 100 mL
1 3561 99 10 023	R1 1 x 800 mL	+	R2 1 x 200 mL
1 3561 99 10 704	R1 8 x 50 mL	+	R2 8 x 12.5 mL
1 3561 99 10 917	R1 8 x 60 mL	+	R2 8 x 15 mL
1 3561 99 10 930	R1 4 x 20 mL	+	R2 2 x 10 mL

Kits for use in conjunction with DiaSys CE applications.

Intended Use

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

Summary

Cholesterol, synthesized by body cells and absorbed with food, is a component of cell membranes and a precursor for steroid hormones and bile acids [1,3]. Cholesterol is transported in plasma via lipoproteins, complexes between lipids and apolipoproteins. Four lipoprotein classes exist: High density lipoproteins (HDL), low density lipoproteins (LDL), very low-density lipoproteins (VLDL) and chylomicrons. These classes show distinct relationship to coronary atherosclerosis. LDL is involved in the cholesterol transport to the peripheral cells, contributing to atherosclerotic plaque formation within the arterial intima and is strongly associated with coronary heart disease (CHD) and related mortality [1-3]. HDL-C has a protective effect impeding plaque formation and shows an inverse relationship to CHD prevalence [4]. In fact, low HDL-C values constitute an independent risk factor. One of the important functions of HDL involves the physiological removal of cholesterol from peripheral tissues and cells, and transport to the liver. The concept that HDL could protect against CHD primarily originated from epidemiological studies of the healthy population, particularly the Framingham study [3-6]. In addition to a number of antioxidant effects, HDL also serves as a powerful mediator of the cellular inflammatory and antithrombotic responses [4]. HDL-particles are macromolecule complexes synthesized by liver and intestine and formed from surface components (HDL particles are assembled in plasma and nascent HDL formation synthesized by the liver and intestine, undergo a dynamic process of assembly and maturation in bloodstream). HDL-particles are released into plasma during lipolysis of lipoproteins rich in triglycerides. Particles consist of an amphipathic lipid monolayer of phospholipids and cholesterol with embedded amphipathic proteins surrounding a core of hydrophobic lipids, mostly cholesteryl esters and triglycerides [3-6]. HDL-C monitoring is highly relevant in cardiovascular risk assessment. Elevated HDL-C levels usually correlate with decreased cardiovascular risk; whereas reduced concentrations of HDL-C, especially in combination with elevated triglycerides are associated with high risk of atherosclerotic heart disease, even at or below recommended LDL-C goals. Preferred screening tests for dyslipidemia or lipid disorders are total cholesterol (TC) and HDL-C but majority of screening guidelines nowadays recommend a full lipid profile including TC, LDL-C, HDL-C and triglycerides [5-8].

Method

Previous HDL-cholesterol determinations were performed by time-consuming precipitation methods or ultracentrifugation (reference method in combination with cholesterol measurement by Abell-Kendall). However, the direct determination of HDL-cholesterol is used in routine [9]. HDL-c direct FS is a homogeneous method for HDL-cholesterol measurement without centrifugation steps. Block polymer detergents protect LDL, VLDL and chylomicrons in a way that only HDL-cholesterol is selectively determined by an enzymatic cholesterol measurement [10].

HDL-cholesterol ester $\xrightarrow{\text{CHE \& CHO}}$ Δ^4-Cholestenon + free fatty acids + H_2O_2

H_2O_2 + 4-Aminoantipyrine + H-DAOS $\xrightarrow{\text{POD}}$ Blue dye + H_2O

The intensity of the formed dye is directly proportional to the cholesterol concentration and is measured photometrically.

Reagents

Components and Concentrations

R1:	Buffer	pH 6.85	20 mmol/L
	Peroxidase (POD)		≥ 2000 U/L
	N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline sodium salt (H-DAOS)		≥ 0.7 mmol/L
R2:	Buffer	pH 8.15	20 mmol/L
	Cholesterol esterase (CHE)		≥ 400 U/L
	Cholesterol oxidase (CHO)		≥ 700 U/L
	Peroxidase (POD)		≥ 15000 U/L
	4-Aminoantipyrine		≥ 1.5 mmol/L

Storage and Stability

Reagents are stable up to the date of expiry indicated on the kit, if stored at 2 – 8°C and contamination is avoided. Do not freeze and protect from light.

The open-vial stability of the reagent is 24 months until expiry date.

Warnings and Precautions

1. Components contained in HDL-c direct FS are classified according to EC regulation 1272/2008 (CLP) as follows:

⚠ Reagent 1: Warning. Contains Mixture of 5-chlorine-2-methyl-2H-isothiazol-3-on and 2-methylen-2H-isothiazol-3-on (3:1). H317 May cause an allergic skin reaction. H412 Harmful to aquatic life with long lasting effects. P261 Avoid breathing vapours. P273 Avoid release to the environment. P280 Wear protective gloves/protective clothing/eye 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. Reagent 2 contains sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
3. The reagents contain material of biological origin. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practice.
4. Acetaminophen and metamizole medication leads to falsely low results in patient samples.
5. In very rare cases, samples of patients with gammopathy might give falsified results [11].
6. In case of product malfunction or altered appearance that could affect the performance, contact the manufacturer.
7. Any serious incident related to the product must be reported to the manufacturer and the competent authority of the Member State where the user and/or patient is located.
8. Please refer to the safety data sheets (SDS) 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.
9. For professional use only.

Waste Management

Refer to local legal requirements for chemical disposal regulations as stated in the relevant SDS to determine the safe disposal.

Warning: Handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Reagent Preparation

The reagents are ready to use.

Materials Required

General laboratory equipment

Specimen

Human serum or lithium heparin plasma

Only use suitable tubes or collection containers for specimen collection and preparation.

When using primary tubes, follow the manufacturer's instructions.

Stability [12]:

2 days	at	20 – 25°C
7 days	at	4 – 8°C
3 months	at	-20°C

Only freeze once. Discard contaminated specimens.

Assay Procedure

Basic settings for BioMajesty® JCA-BM6010/C

Wavelength	596/694 nm
Temperature	37°C
Measurement	Endpoint
Sample/calibrator	1.0 µL
Reagent 1	80 µL
Reagent 2	20 µL
Addition reagent 2	Cycle 19 (286 s)
Absorbance 1	Cycle 17/18 (231 s/244 s)
Absorbance 2	Cycle 41/42 (586 s/600 s)
Calibration	Linear

Calculation

With Calibrator

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

Conversion Factor

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

Calibrators and Controls

DiaSys TruCal Lipid is recommended for calibration. Calibrator values have been made traceable to a commercially available assay, which is standardized against the designated CDC reference method (ultracentrifugation method). Use DiaSys TruLab L Level 1 and Level 2 for internal quality control. All target values of the controls are traceable to DiaSys reagent/calibrator system. Quality control must be performed after calibration. Control intervals and limits have to be adapted to the individual requirements of each laboratory. Results must be within the defined ranges. Follow the relevant legal requirements and guidelines. 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

Data evaluated on BioMajesty® JCA-BM6010/C

Measuring range from 3 mg/dL up to 200 mg/dL. Linearity ≤ 11 mg/dL is given within ± 15%, at > 11 mg/dL within ± 10%. When values exceed this range samples should be diluted 1 + 2 with NaCl solution (9 g/L) and the result multiplied by 3.	
Limit of detection**	3 mg/dL
Limit of quantitation**	3 mg/dL

Interference by	**Interferences ≤ 10% up to**	**Analyte concentration [mg/dL]**
Ascorbic acid	60 mg/dL	35.3
	60 mg/dL	79.1
Bilirubin (conjugated)	40 mg/dL	38.9
	40 mg/dL	78.9
Bilirubin (unconjugated)	60 mg/dL	42.7
	60 mg/dL	81.4
Hemolysis	800 mg/dL	32.9
	1000 mg/dL	71.9
Lipemia (triglycerides)	1000 mg/dL	37.7
N-acetylcysteine (NAC)	1700 mg/L	35.8
	1700 mg/L	72.3
For further information on interfering substances, refer to the literature [13,14].		

Precision			
Repeatability (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [mg/dL]	17.9	43.7	184
CV [%]	1.52	1.29	0.661
Within-laboratory (n=80)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [mg/dL]	17.9	44.7	186
CV [%]	2.26	1.86	1.80

Method comparison (n=115)	
Test x	Competitor HDL-C (cobas c 501)
Test y	DiaSys HDL-c direct FS (BioMajesty® JCA-BM6010/C)
Slope	1.01
Intercept	-0.118 mg/dL
Coefficient of correlation	0.998

** according to CLSI document EP17-A2, Vol. 32, No. 8

Reference Range [15]

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 the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature

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15. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the 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). JAMA. 2001;285(19):2486-2497.

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IVD CE

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