BioMajesty®

HDL-c direct FS*

Order Information

Cat. No.
1 3561 99 10 962

Kit size
Σ 1890 (R1: 6 x 315, R2: 6 x 315)

Intended Use

Diagnostic reagent for quantitative in vitro determination of HDL-C (high density lipoprotein cholesterol) in human serum or heparin plasma on automated BioMajesty® JCA-BM6010/C.

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. 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. HDL-C has a protective effect impeding plaque formation and shows an inverse relationship to CHD prevalence. 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, in particular the Framingham study. In addition to a number of antioxidant effects, HDL also serves as a powerful mediator of the cellular inflammatory and antithrombotic responses. HDL-particles are macromolecule complexes synthesized by liver and intestine and formed from surface components. 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. 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 the majority of screening guidelines nowadays recommend a full lipid profile including TC, LDL-C, HDL-C and triglycerides. [1-8]

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 in-use stability of the reagent is 24 months.

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. P280 Wear protective gloves/protective clothing/eye protection. P302+P352 IF ON SKIN: Wash with plenty of water/soap.

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. The bottles are placed directly into the reagent rotor.

Materials Required

General laboratory equipment

BioMajesty®

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.

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. 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

Measuring range up to 200 mg/dL.
In case of higher concentrations re-measure samples after manual dilution with NaCl solution (9 g/L) or use rerun function.

Limit of detection**	3 mg/dL
Onboard stability	12 weeks
Calibration stability	12 weeks

Interfering substance	Interferences ≤ 10% up to	Analyte concentration [mg/dL]
Ascorbic acid	60 mg/dL	35.3
	60 mg/dL	79.1
Bilirubin (conjugated)	50 mg/dL	38.9
	50 mg/dL	78.9
Bilirubin (unconjugated)	60 mg/dL	42.7
	60 mg/dL	81.4
Hemoglobin	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 Young DS [13,14].

Precision			
Within run (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [mg/dL]	17.9	43.7	184
CV [%]	1.52	1.29	0.661
Total precision CLSI (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

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

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

1. Grundy SM, Stone NJ, Bailey AL, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol. J Am Coll Cardiol. 2019;73(24):e285–e350.
2. Gordon DJ, Probstfield JL, Garrison RJ, et al. High density lipoprotein cholesterol and cardiovascular disease. Four Prospective American Studies. Circulation. 1989;79:8-15.
3. Favari E, Chroni A, Tietge UJF et al. Cholesterol Efflux and Reverse Cholesterol Transport. In: Eckardstein A and Kardassis D, editors. High Density Lipoproteins: From Biological Understanding to Clinical Exploitation. Springer; 2015. page 181-206.
4. Barter PJ, Nicholls S, Rye KA, et al. Antiinflammatory properties of HDL. Circulation research. 2004;95:764-772.
5. Lee JS, Chang PY, Zhang Y, et al. Triglyceride and HDL-C Dyslipidemia and Risks of Coronary Heart Disease and Ischemic Stroke by Glycemic Dysregulation Status: The Strong Heart Study. Diabetes Care. 2017;40:529-537.
6. Chapman MJ, Ginsberg HN, Amarenco P, et al. Triglyceride-rich lipoproteins and high-density lipoprotein cholesterol in patients at high risk of cardiovascular disease: evidence and guidance for management. European heart journal volume. 2011;32:1345-61.
7. Rifai N, Warnwick GR. Lipids, Lipoproteins, Apolipoproteins, and Other Cardiovascular Risk Factors. In: Burtis CA, Ashwood ER and Burns DE, editors. Tietz Textbook of Clinical Chemistry. 4th ed. Missoury: Elsevier Saunders company; 2006. page 903-981.
8. Task Force Report of European and other Societies on Coronary Prevention. Recommendation of the Second Joint Task Force of European and other Societies on Coronary Prevention. Prevention of coronary heart disease in clinical practice. European Heart Journal; 1998. Report No.: hj981243.
9. Langlois MR, Blaton VH. Historical milestones in measurement of HDLcholesterol: Impact on clinical and laboratory practice. Clin Chimica Acta. 2006;369:168-178.
10. Miida T, Nishimura K, Okamura T, et al. Validation of homogeneous assays for HDL-cholesterol using fresh samples from healthy and diseased subjects. Atherosclerosis. 2014; 233(1):253-9.
11. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed. 2007;45(9):1240-1243.
12. Guder WG, da Fonseca-Wollheim F, Heil W, et al. The Quality of Diagnostic Samples. 3rd ed. Darmstadt: GIT Verlag; 2010. p. 22-3.

13. 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.
14. Young DS. Effects on Clinical Laboratory Tests - Drugs Disease, Herbs & Natural Products [Internet]. AACC Press and John Wiley and Sons, Inc; 2020 [cited 2020 May]. Available from: https://clinfx.wiley.com/ aaccweb/aacc/
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.

Additions and/or changes in the document are highlighted in grey. For deletions, please refer to the customer information for the corresponding edition number of the package inserts.

IVD CE

DiaSys Diagnostic Systems GmbH
Alte Strasse 9 65558 Holzheim
Germany
www.diasys-diagnostics.com

* Fluid Stable

BioMajesty

HDL-c direct FS

Chemistry code 10 356

Application for serum and plasma samples

This application was set up and evaluated by DiaSys. It is based on the standard equipment at that time and does not apply to any equipment modifications undertaken by unqualified personnel.

Analytical Conditions	
R1 volume	80
R2e volume	0
R2 volume	20
R1 diluent vol	0
R2e diluent vol	0
R2 diluent vol	0
Sample vol (S)	1.0
Sample vol (U)	1.0
Reagent 1 mix	weak
Reagent 2e mix	weak
Reagent 2 mix	weak
Reaction time	10

Sub-analy. Conditions	
Name	HDL-C
Digits	2
M-wave L.	596
S-wave.L	694
Analy.mthd.	EPA
Calc.mthd.	STD
Qualit. judge	No

Analysis Test Condition Setting (M)		
Sample Type	**Serum**	**Urine**
Reac. sample vol.	1.0	1.0
Diluent method	No dil	No dil
Undil. sample vol.	0	0
Diluent volume	0	0
Diluent position	0	0

entered by user

Endpoint method	
Re.absorb (u)	9.999
Re. Absorb (d)	-9.999

Calculation Method Setting	
M-DET.P.I	0
M-DET.P.m	41
M-DET.P.n	42
S-DET.P.p	17
S-DET.P.r	18
Check D.P.I.	0
Limit value	0.003
Variance	10
Reac.type	Inc

Reaction Rate Method	
Cycle	2
Factor	2
E2 corre	Not do
Blank (u)	9.999
Blank (d)	-9.999
Sample (u)	9.999
Sample (d)	-9.999

Standards Setting	
FV	#
BLK H	9.999
BLK L	-9.999
STD H	9.999
STD L	-9.999