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

Intended Use

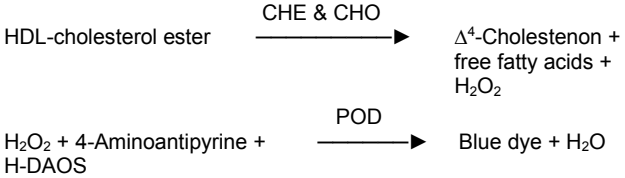
Diagnostic reagent for quantitative in vitro determination of HDL-C (high density lipoprotein cholesterol) in human serum and heparin plasma on automated photometric systems.

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. 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].



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. Protect the reagents from light.

Warnings and Precautions

1. ⚠ 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 animal material. Handle the product as potentially infectious according to universal precautions and good 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. 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.
7. For professional use only.

Waste Management

Refer to local legal requirements.

Reagent Preparation

The reagents are ready to use.

Materials Required

General laboratory equipment

Specimen

Human serum and heparin plasma (Lithium)

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. TruCal Lipid calibrator values have been made traceable to NIST-SRM®-1951 Level 2. Use DiaSys TruLab L Level 1 and Level 2 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

Data evaluated on BioMajesty® JCA-BM6010/C

Exemplary data for serum mentioned below may slightly differ in case of deviating measurement conditions.

Measuring range up to 200 mg/dL. 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

Interfering substance	**Interferences ≤ 10% up to**
Ascorbic acid	60 mg/dL
Bilirubin (conjugated)	50 mg/dL
Bilirubin (unconjugated)	60 mg/dL
Hemoglobin	800 mg/dL
Lipemia (triglycerides)	1000 mg/dL
N-acetylcysteine(NAC)	1700 mg/L

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=146)	
Test x	Competitor HDL-C
Test y	DiaSys HDL-c direct FS
Slope	1.08
Intercept	–1.05 mg/dL
Coefficient of correlation	0.987

** 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

1. Grundy SM et al. AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation. 2018; 138: e1082-e1143.
2. Gordon DJ, Probstfield JL, Garrison RJ, et al. High density lipoprotein cholesterol and cardiovascular disease. Four Prospective American Studes. Circulation. 1989; 79: 8-15.
3. Favari E, Chroni A, Tietge UJF et al. High Density Lipoproteins: From Biological Understanding to Clinical Exploitation. Springer Verlag; Volume 224, 2015; p. 181-206.
4. Barter PJ, Nicholls S, Rye KA, Anantha-ramaiah G, Navab M and Fogelman AM (2004). Antiinflammatory properties of HDL. Circ. Res. 95.
5. Lee JS, Chang P-Y, Zhang Y, Kizer JR, Best LG and Howard BV. 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, M John 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 32, 11 (2011): 1345-61. 764-772.
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. p. 903-981.
8. 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.
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, Zawta B et al. The Quality of Diagnostic Samples.1st ed. Darmstadt: GIT Verlag; 2001; 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, https://clinfx.wiley.com/aaccweb/aacc/, accessed on May 2020. Published by AACC Press and John Wiley and Sons, Inc..
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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* Fluid Stable