LDL-c direct FS*

Order Information

Cat. No.	Kit size		
1 4131 99 10 021	R1 5 x 20 mL	+	R2 **1 x 25 mL**
1 4131 99 10 026	R1 5 x 80 mL	+	R2 **1 x 100 mL**
1 4131 99 10 930	R1 4 x 20 mL	+	R2 **2 x 10 mL**

Intended Use

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

Summary

Cholesterol is usually obtained from the intestinal absorption of dietary and biliary cholesterol but can also be synthesized de novo in various tissues, predominantly in liver and intestine. An adult on a low-cholesterol diet typically synthesizes about 800 mg of cholesterol per day. Cholesterol is essential for all cells and is used extensively as a major structural component of cell membranes and as substrate for the synthesis of bile acids, vitamin D, and sex hormones (estradiol, progesterone, androsterone and testosterone). Cholesterol is insoluble in water and, therefore, must be transported bound to proteins. Lipoproteins are complex particles with a central core containing cholesterol esters and triglycerides (TG) surrounded by free cholesterol, phospholipids, and apolipoproteins, which facilitate lipoprotein formation and function. Plasma lipoproteins can be divided into different classes based on size, lipid composition, and apolipoproteins; the four major classes are: Chylomicrons, very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL). Low-density lipoproteins are derived from VLDL and IDL (Intermediate Density Lipoprotein) in plasma and contain a large amount of cholesterol and cholesterol esters. The principal role of LDL is to deliver these two forms of cholesterol to peripheral tissues. At least two-thirds of circulating cholesterol reside in LDL. Evidence from epidemiologic, genetic, and clinical intervention studies has shown that LDL is causal in the process of developing atherosclerotic cardiovascular disease (ASCVD). High LDL-C is one of the major risk factors that contribute to the formation of atherosclerotic plaques within the arterial intima and is strongly associated with coronary heart disease (CHD) and related mortality. Results of recent clinical studies on lowering LDL-C indicate continued benefits at low concentrations. A direct linear relationship between the pharmacological lowering of LDL-C and the relative risk reduction in cardiovascular events has been observed for three different drug classes: statins, ezetimibe and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors. The standard lipid panel represents a well-established platform to assess risk, but this panel alone may be insufficient and/or misleading. By now, the majority of screening guidelines recommend the measurement of a full lipid profile including total cholesterol (TC), LDL-C, HDL-cholesterol (HDL-C) and TG. [1-6]

Method

Different methods exist to determine LDL-C. The reference method is the ultracentrifugation, which is tedious and technically demanding, therefore, not suitable for routine. A common approach to determine LDL-C in clinical laboratory is the Friedewald calculation, which estimates LDL-C from measurements of TC, triglycerides (TG), and HDL-C but the method only approximates LDL-C and is subject to well-established limitations. At the end of the last century, homogeneous LDL-C methods for fully automated determination were introduced. Those methods enable direct determination of LDL-cholesterol and show other advantages compared to previously used methods. LDL-c direct FS is a homogeneous method without centrifugation steps for direct measurement of LDL-cholesterol. Block polymer detergents protect HDL, VLDL and chylomicrons in a way that only LDL-cholesterol is selectively determined by an enzymatic cholesterol measurement. [7]

LDL-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 6.65	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)		≥ 2000 U/L
	Cholesterol oxidase (CHO)		≥ 2000 U/L
	Peroxidase (POD)		≥ 15000 U/L
	4-Aminoantipyrine (4-AA)		≥ 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 18 months.

Warnings and Precautions

1. Components contained in LDL-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. Artificial lipid mixtures (e.g. Intralipid®) may interfere with the test. Serum samples from patients treated with such solutions should not be used.
5. Determination of samples from patients with a rare type of Hyperlipoproteinemia (Hyperlipoproteinemia Type III) may lead to false results.
6. In very rare cases, samples of patients with gammopathy might give falsified results [8].
7. Acetaminophen and metamizole medication leads to falsely low results in patient samples.
8. In case of product malfunction or altered appearance that could affect the performance, contact the manufacturer.
9. 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.
10. 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.
11. 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 heparin plasma

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

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

Stability [9,10,11]:

1 day	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 (bichromatic)
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

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

Conversion Factor

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

Calibrators and Controls

DiaSys TruCal Lipid is recommended for calibration. Calibrator values have been made traceable to NIST SRM 1951c Level 2. 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

Data evaluated on BioMajesty® JCA-BM6010/C

Measuring range up to 500 mg/dL. When values exceed this range, samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the result multiplied by 2.

Limit of detection**	4 mg/dL

Interfering substance	Interferences ≤ 9% up to	Analyte concentration [mg/dL]
Ascorbic acid	500 mg/dL	74.2
	500 mg/dL	168
Bilirubin (conjugated)	60 mg/dL	86.4
	60 mg/dL	157
Bilirubin (unconjugated)	60 mg/dL	87.1
	60 mg/dL	157
Hemoglobin	1000 mg/dL	76.7
	1000 mg/dL	159
Lipemia (triglycerides)	1500 mg/dL	77.4
	1500 mg/dL	163
N-acetylcysteine (NAC)	1600 mg/L	70.9
	1600 mg/L	161

For further information on interfering substances, refer to Young DS [12,13].

Precision			
Within run (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [mg/dL]	90.8	149	433
CV [%]	0.912	0.909	0.582
Between day (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [mg/dL]	89.1	143	419
CV [%]	1.68	0.971	1.17

Method comparison (n=118)	
Test x	Competitor LDL-C (cobas c 501)
Test y	DiaSys LDL-c direct FS (BioMajesty® JCA-BM6010/C)
Slope	0.997
Intercept	–1.17 mg/dL
Coefficient of correlation	0.997

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

Reference Range [14]

Desirable	< 100 mg/dL	< 2.59 mmol/L
Above optimal	100 – 129 mg/dL	2.59 – 3.34 mmol/L
Borderline high risk	130 – 159 mg/dL	3.37 – 4.12 mmol/L
High risk	160 – 189 mg/dL	4.14 – 4.89 mmol/L
Very high risk	> 190 mg/dL	> 4.92 mmol/L

Patient risk classification, management and treatment therapies are described in the 2018 AHA/ACC Guideline on the Management of Blood Cholesterol [15].

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

Clinical Interpretation

The lipid guidelines of the European Society of Cardiology (ESC)/European Atherosclerosis Society (EAS) 2019 have set targets for the reduction of low-density lipoproteins (LDL) as follows:

Very high-risk patients:

≥ 50% LDL-C reduction from baseline and an absolute LDL-C treatment goal of < 1.4 mmol/L (< 55 mg/dL)

High risk patients:

≥ 50% LDL-C reduction and a LDL-C goal of < 1.8 mmol/L (< 70 mg/dL)

Literature

1. Berg JM, Tymoczko JL, Stryer L. New York: W H Freeman; 2015. Biochemistry. 8th edition. Section 26.3 The Complex Regulation of Cholesterol Biosynthesis Takes Place at Several Levels, Page 779 – 788.
2. Berg JM, Tymoczko JL, Stryer L. New York: W H Freeman; 2015. Biochemistry. 8th edition. S Section 26.4 Important Derivatives of Cholesterol Include Bile Salts and Steroid Hormones, page 788 – 795.
3. Feingold KR, Grunfeld C. Introduction to Lipids and Lipoproteins. [Updated 2018 Feb 2]. In: Feingold KR, Anawalt B, Boyce A, et al., editors. Endotext [Internet]. South Dartmouth

(MA): MDText.com, Inc.; 2000-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK305896/
4. Huff, T.; Jialal, I.I. Physiology, Cholesterol; StatPearls Publishing: Orlando, FL, USA, 2017.
5. Ference BA, Ginsberg HN et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J 2017;38: 2459–2472.
6. Pirillo A., Norata G.D., Catapano A.L. (2020) LDL-Cholesterol-Lowering Therapy. In: Handbook of Experimental Pharmacology. Springer, Berlin, Heidelberg.
7. Nauck M, Warnwick GR, Rifai N. Methods for measurement of LDL-cholesterol: a critical assessment of direct measurement by homogeneous assays versus calculation. Clin Chem 2002;48:236-54.
8. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007;45(9):1240-1243.
9. WHO Publication: Use of anticoagulants in diagnostic laboratory. investigations, WHO/DIL/LAB/99.1 Rev.2:Jan 2002.
10. Jansen EHLM, Beekhof PK, Schenk E. Long Term Stability of Lipid Metabolism in Frozen Human Serum: Triglycerides, Free Fatty Acids, Total-, HDL- and LDL-cholesterol, Apolipoprotein-A1 and B. J Mol Biomark Diagn 2014;5:4.
11. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001; p. 22-3.
12. 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.
13. Young DS. Effects on Clinical Laboratory Tests - Drugs Disease, Herbs & Natural Products, https://clinfx.wiley.com/aaccweb/aacc/, accessed in February 2021. Published by AACC Press and John Wiley and Sons, Inc.
14. 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.
15. Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, et al. 2018 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. J Am Coll Cardiol. 2018;73(24):e285-e350.

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