Instructions for Use
For *in vitro* diagnostic use

LDL-C DIRETO

LDL-C Direct

Anvisa 80115310278

ORDER INFORMATION

Cat. No.	Kit size	
1170075K	R1 3 x 20 mL	+ R2 1 x 15 mL
1170050K	R1 2 x 20 mL	+ R2 1 x 10 mL
1170075M	R1 3 x 20 mL	+ R2 1 x 15 mL
1170250K	R1 1 x 200 mL	+ R2 1 x 50 mL
1170200R	R1 4 x 38,6 mL	+ R2 4 x 11,4 mL
1170050MK	R1 1 x 40 mL	+ R2 1 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), lowdensity 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 can be found in LDL. Evidence from epidemiologic, genetic, and clinical intervention studies have 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 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 have been 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 LDLcholesterol. Block polymer detergents protect HDL, VLDL and chylomicrons in a way that only LDL-cholesterol is selectively determined by an enzymatic cholesterol measurement.

PRINCIPLE

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-hidroxi-3-sulfopropil)-3.5-dimetoxyaniline (H-DAOS) sodium salt		≥ 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!

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 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. Acetaminophen and metamizole medication leads to falsely low results in patient samples.
7. In very rare cases, samples of patients with gammopathy might give falsified results [11].
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

Follow the requirements of the current guidelines about technical regulation for the management of healthcare service waste, as well as other equivalent biosafety practices.

REAGENT PREPARATION

The reagents are ready to use.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. General laboratory equipment.

SPECIMEN

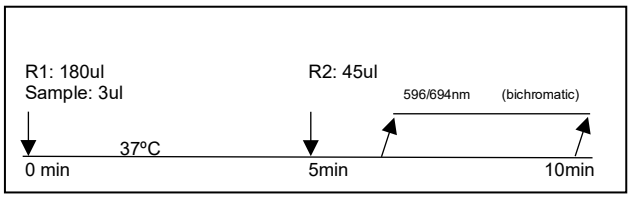
Human serum or heparin plasma

Stability [9-11]:	1 day	at	20 - 25 ºC
	7 days	at	4 - 8 ºC
	12 months	at	-20 ºC

Discard contaminated specimens.
Only freeze once.

ASSAY PROCEDURE

The standard procedure is represented below as a general example for utilization:



Settings:

Wavelength	596 / 694 nm (bichromatic)
Temperature	37° C
Measurement	Endpoint
Sample/Calibrator	3 ul
Reagent 1	180 ul
Reagent 2	45 ul
Addition Reagent 2	5 min (300s)
Absorbance 1	(~ 286s) Must be before 5 min
Absorbance 2	10 min (600s)
Calibration	Linear

CALCULATION

With Calibrator

$$\text{LDL} - \text{C}\ [\text{mg/dl}] = \frac{\Delta\text{ASample}}{\Delta\text{ACalibrator}} \times \text{Conc. Calibrator}\ [\text{mg/dl}]$$

CALIBRATORS AND CONTROLS

For calibration on automated photometric systems, Kovalent Topkal HDL/LDL is recommended for calibration. Use Topkon L, Topkon N or Topkon P from Kovalent for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

WARRANTY

These instructions for use should be read carefully before using the product and the information contained therein should be strictly adhered to. The reliability of the test results cannot be guaranteed if the instructions are not followed.

PERFORMANCE CHARACTERISTICS

Data evaluated on BioMajesty®JCA-BM6010/C

The below-mentioned data may slightly differ when submitted to distinct measurement conditions.

Measuring range

The assay was designed to measure concentrations of LDL-C 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.

Specificity/ Interferences

Interference by	Interference ≤ 9% up to
Ascorbic acid	500 mg/dL
Bilirubin (conjugated)	60 mg/dL
Bilirubin (unconjugated)	60 mg/dL
Hemolysis	1000 mg/dL
Lipemia (triglycerides)	1500 mg/dL
N-acetilcysteine (NAC)	1600 mg/L

For further information on interfering substances, refer to the literature [12,13].

Sensitivity / Limit of Detection

The limit of detection is 4 mg/dL*.

Precision

Within-assay Precision n = 20	Mean [mg/dL]	CV [%]
Sample 1	90.8	0.912
Sample 2	149	0.909
Sample 3	433	0.582

Between-days Precision n = 20	Mean [mg/dL]	CV [%]
Sample 1	89.1	1.68
Sample 2	143	0.971
Sample 3	419	1.17

Method Comparison

Method Comparison (n=118)	
Test x	Competitor LDL-C Cobas c 501
Test y	DiaSys LDL-c Direct FS BioMajesty® JCA-BM6010C
Slope	0.997
Intercept	-1.17
Coefficient of correlation	0.997

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

Conversion Factor

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

REFERENCE RANGE [14]

Desirable	< 100 mg/dL	2.59 mmol/L
Above ideal	100 – 129 mg/dL	2.59 – 3.34 mmol/L
Boderline 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

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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.

CONSUMER INFORMATION

Symbols used:

	Manufacturer
	Temperature limit
	In vitro diagnostic device
	Caution
	Operating instructions
	Recycling material
	Do not discard directly into the environment
	Batch code
	Date of manufacture
	Use by date
	Biological hazards
	Highly toxic
	Corrosive
	Harmful

Manufacturer:

Kovalent do Brasil Ltda.
Rua Cristóvão Sardinha, 110 – Jd. Bom Retiro
São Gonçalo – RJ – CEP 24722-414 - Brasil
www.kovalent.com.br
CNPJ: 04.842.199/0001-56

Customer Service: sac@kovalent.com.br - (21) 3907-2534 / 0800 015 1414

Expiration date and Lot no.: See label