HDL-C DIRETO

HDL-C Direct

Anvisa 80115310267

ORDER INFORMATION

Cat. No.	Kit size
1160075K	R1: 3 x 20 mL + R2: 1 x 15 mL
1160075M	R1: 3 x 20 mL + R2: 1 x 15 mL
1160250K	R1: 1 x 200 mL + R2: 1 x 50 mL
1160200R	R1: 4 x 38,6 mL + R2: 4 x 11,4 mL
1160050MK	R1: 1 x 40 mL + R2: 1 x 10 mL

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. 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. Even if total cholesterol (TC) is in a normality range, a high LDL-cholesterol concentration indicates high risk.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, particularly 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 [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. 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 [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 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].

PRINCIPLE

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, protected from light and if contamination is avoided. Do not freeze the reagents!

WANINGS 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. 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 (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.
7. 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 PROVIDED

1. General laboratory equipment.

SPECIMEN

Human serum or lithium heparin plasma

Stability [12]:	2 days	at	20 - 25 ºC
	7 days	at	4 - 8 ºC
	3 months	at	-20 ºC

Discard contaminated specimens.
Only freeze once.

ASSAY PROCEDURE

Applications for automatic systems are available upon request or on our website: www.kovalent.com.br

The manual procedure may differ slightly from the applications for automated systems.

Wavelength	600 nm / 700 nm (Bichromatic Dosage)
Optical path	1 cm
Temperature	37 ºC
Measurement	Against reagent blank

	Blank	Sample or calibrator
Sample or calibrator	-	2.4 µ L
Distilled water	2.4 µL	-
Reagent 1	240 µL	240 µL

Mix, incubate for 5 minutes at 37°C, read the absorbance A1, then add:		
Reagent 2	60 µL	60 µL
Mix, incubate for 5 minutes at 37°C and then read the absorbance A2.		

ΔA= [(A2 – 0.8 A1) sample or calibrator] – [(A2 – 0.8 A1) blank]

The factor 0.8 compensates for the decrease in absorbance by the addition of reagent 2.

The factor is calculated as follows:
(Sample + R1) / Total Volume

CALCULATION

With Calibrator

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

Conversion Factor

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

CALIBRATORS AND CONTROLS

For calibration in automated photometric systems, Kovalent Topkal HDL/LDL calibrator is recommended. Use Kovalent Topkon N, P and L 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 data mentioned below may differ slightly under conditions of divergent measurements.

Measuring range

The assay is designed to determine HDL-C concentrations within a measurement range of 3 - 200 mg/dL. When the values exceed this range, the samples should be diluted 1 + 2 with NaCl solution (9 g/L) and the result multiplied by 3.

Specificity / Interferences

Interference substance	Interference ≤ 10% up to
Ascorbic acid	60 mg/dL
Direct bilirubin	50 mg/dL
Indirect bilirubin	60 mg/dL
Hemoglobin	800 mg/dL
Lipemia (triglycerides)	1000 mg/dL
N-acetylcysteine (NAC)	1700 mg/dL

For further information on interfering substances, refer to the literature [13,14].

Sensitivity / Limit of Detection**

The lowest detection limit is 3 mg/dL.

Precision

Intra-assay precision n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	17.9	0.271	1.52
Sample 2	43.7	0.563	1.29
Sample 3	184	1.22	0.661

Total precision CLSI n = 80	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	17.9	0.405	2.26
Sample 2	44.7	0.832	1.86
Sample 3	186	3.34	1.80

Method comparison

Method comparison (n=146)	
Test x	Commercially available assay
Test y	HDL-C Direto
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 its 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.
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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. 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.

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

Kit sizes variations on demand:

Anvisa No.	Kit size
80115310267	R1: 4 x 40 mL + R2: 4 x 10 mL
80115310267	R1: 2 x 40 mL + R2: 2 x 10 mL
80115310267	R1: 3 x 40 mL + R2: 3 x 10 mL

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Expiration date and Lot no.: See label