Instructions for Use

For *in vitro* diagnostic use

COLESTEROL WS

Cholesterol WS

Anvisa 80115310195

ORDER INFORMATION

Cat. No.	Kit size
1020500KWS	R: 2 x 250 mL
1020180MWS	R: 6 x 30 mL
1020174.4RWS	R: 4 x 43,6 mL
1020120MKWS	R: 3 x 40 mL

INTENDED USE

Diagnostic reagent for quantitative *in vitro* determination of Cholesterol in human serum or plasma on photometric systems.

SUMMARY [1,2]

Cholesterol is a component of cell membranes and a precursor for steroid hormones and bile acids synthesized by body cells and absorbed with food. Cholesterol is transported in plasma via lipoproteins, namely complexes between lipids and apolipoproteins. Four classes of lipoproteins exist: high density lipoproteins (HDL), low density lipoproteins (LDL), very low-density lipoproteins (VLDL) and chylomicrons. While LDL is involved in the cholesterol transport to the peripheral cells, HDL is responsible for the cholesterol uptake from the cells. The four different lipoprotein classes show distinct relationship to coronary atherosclerosis. LDL cholesterol (LDLC) contributes to atherosclerotic plaque formation within the arterial intima and is strongly associated with coronary heart disease (CHD) and related mortality. Even with total cholesterol within the normal range, an increased concentration of LDL-C 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. The determination of the individual total cholesterol (TC) level is used for screening purposes while for a better risk assessment it is necessary to measure additionally HDL-C and LDL-C.
In the last few years several controlled clinical trials using diet, lifestyle changes and / or different drugs (especially HMG CoA reductase inhibitors [statins]) have demonstrated that lowering total cholesterol and LDL-C levels reduce drastically the CHD risk.

METHOD

"CHOD-PAP": enzymatic photometric test.

PRINCIPLE

Determination of cholesterol after enzymatic hydrolysis and oxidation[3,4]. The colorimetric indicator is quinoneimine, which is generated from 4-aminoantipyrine and phenol by hydrogen peroxide under the catalytic action of peroxidase (Trinder's reaction) [3].

$$\text{Cholesterol ester} + H_2O \xrightarrow{CHE} Cholesterol + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHO}} \text{Cholesterol} - 3 - \text{one} + H_2O_2$$

$$2H_2O_2 + 4 - \text{Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + 4H_2O$$

REAGENTS

Components and Concentrations

Monoreagent

Buffer	pH 6,7	< 100 mmol/L
Phenol		5 mmol/L
4–Aminoantipyrine		< 1 mmol/L
Lipoprotein Lipase		< 500 U/L
Cholesterol Oxidase (CHO)		<300U/L
Peroxidase (POD)		<10KU/L

STORAGE AND STABILITY

Reagent is stable up to the date of expiry indicated on the kit, if stored at 2 - 8°C and contamination is avoided.
Note: Measurement is not influenced by occasionally occurring color changes, as long as the absorbance of the reagent is < 0.3 to 546 nm.

WARNING AND PRECAUTIONS

1. The reagent contains sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. Sample: Beware: Harmful when ingested. It can provoke allergic reactions when in contact with skin. It can cause serious reactions to eyes and if the reaction persists it is recommended to seek medical assistance. Use gloves, clothes, glasses and protective masks. Wash hands and face after handling samples. In case of contact with skin, wash it with plenty of water and soap.
3. In very rare cases, samples of patients with gammopathy might give falsified results [8].
4. N-acetylcysteine (NAC), acetaminophen and metamizole medication leads to falsely low results in patient samples.
5. 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.
6. 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.

REAGENTS PREPARATION

The reagent is ready to use.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. NaCl Solution 9 g/L.
2. General laboratory equipment.

SPECIMEN

Human serum, heparin plasma or EDTA-plasma
Stability [6]: 7 days at 20 - 25 ºC
7 days at 4 - 8 ºC
3 months at -20 ºC
Only freeze once. Discard contaminated specimens.

ASSAY PROCEDURE

Application sheets for automated systems are available on request.

Wavelength	500 nm, Hg 546 nm
Optical path	1 cm
Temperature	20 - 25 ºC/ 37°C
Measurement	Against reagent blank

	Blank	Sample or calibrator
Sample or calibrator	-	10 µL
Distilled water	10 µL	-
Reagent	1000 µL	1000 µL

Mix and incubate for 5 min at 37°C or 10 min at 20-25°C. Read absorbance against blank reagent within 60 min.

CALCULATION

With Calibrator

$$\text{Cholesterol [mg/dL]} = \frac{\text{A Sample}}{\text{A Cal.}} \text{ x Conc. Cal [mg/dL]}$$

Conversion Factor

Cholesterol [mg/dL] x 0.02586 = Cholesterol [mmol/L]

CALIBRATORS AND CONTROLS

For calibration in automated photometric systems, Kovalent Topkal U 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

Measuring Range

The test has been developed to determine concentrations of cholesterol within a measuring range from 3 – 750 mg/dL (0.08 - 19.4 mmol/L). When the values exceed this range, samples must be diluted 1 + 4 with NaCl solution (9 g/L) and the result is multiplied by 5.

Specificity / Interferences

No interference is observed by ascorbic acid up to 5 mg/dL, bilirubin up to 20 mg/dL, hemoglobin up to 200 mg/dL and no lipemia up to 2000 mg/dL triglycerides. For more information on interfering substances, see Young DS [7].

Sensitivity / Limit of Detection

The limit of detection is 3 mg/dL (0.08 mmol/L).

Precision

Within-run precision n = 10	Mean [mg/dL]	SD [mg/dL]	CV [%]
Normal Control	143.80	1.55	1.08
Pathological Control	211.6	3.27	1.55

Inter-assay precision n = 9	Mean [mg/dL]	SD [mg/dL]	CV [%]
Normal Control	151.20	3.92	2.59
Pathological Control	213.10	6.89	3.23

Method Comparison

A comparison of Kovalent's Colesterol WS to a commercially available assay (x) using 30 samples gave the following results:
$y = 1.0025 x + 0.7966$; $R^2 = 0.9988$.

REFERENCE VALUES [5]

	[mg/dL]	[mmol/L]
Desirable	≤ 200	5.2
Borderline high risk	200 - 240	5.2 – 6.2
High risk	> 240	> 6.2

Each laboratory should verify that reference values can be used in its own patient population and determine its own reference values, if necessary.

CLINICAL INTERPRETATION

The European Task Force on Coronary Prevention recommends lowering TC concentration to less than 190 mg/dL (5.0 mmol/L) and LDL-cholesterol to less than 115 mg/dL (3.0 mmol/L)[2].

LITERATURE

1. Rifai N, Bachorik PS, Albers JJ. Lipids, lipoproteins and apolipoproteins. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 809-61.
2. 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.
3. Artiss JD, Zak B. Measurement of cholesterol concentration. In: Rifai N, Warnick GR, Dominiczak MH, eds. Handbook of lipoprotein testing. Washington: AACC Press, 1997:99-114.
4. Deeg R, Ziegenhorn J. Kinetic enzymatic method for automated determination of total cholesterol in serum. Clin Chem 1983; 29:1798-802.
5. Schaefer EJ, McNamara J. Overview of the diagnosis and treatment of lipid disorders. In: Rifai N, Warnick GR, Dominiczak MH, eds. Handbook of lipoprotein testing. Washington: AACC press, 1997:25-48.
6. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001. p. 22 – 3.
7. 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.
8. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007;45(9):1240-1243.

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
80115310195	R: 1 x 250 mL
80115310195	R: 1 x 1000 mL
80115310195	R: 2 x 50 mL
80115310195	R: 4 x 35 mL
80115310195	R: 4 x 50 mL
80115310195	R: 4 x 59,4 mL
80115310195	R: 6 x 39,8 mL
80115310195	R: 6 x 100 mL
80115310195	R: 8 x 50 mL
80115310195	R: 10 x 60 mL
80115310195	R: 12 x 25 mL
80115310195	R: 2 x 20 mL
80115310195	R: 5 x 40 mL

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

Expiration date and Lot no.: See label