ROBONIK®

CE

prietest™ ...Clinical Chemistry Reagents

CHOLESTEROL

In vitro diagnostic test kit, for professional use only

INTENDED USE : in vitro determination of concentration of cholesterol in serum or plasma on photometric systems.

ORDERING INFORMATION

Pack Size	Cat No.
1 X 50 ml	CHO 01 50
2 X 50 ml	CHO 02 50
4 X 50 ml	CHO 04 50
5 X 100 ml	CHO 05 100

CLINICAL SIGNIFICANCE :

Cholesterol is both coming from food and synthesized by the human body, mainly in hepatic and intestinal cells. 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. There are four classes of lipoproteins: high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL) and chylomicrons. 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-Cholesterol and LDL-Cholesterol.

METHOD :

"CHOD-PAP": enzymatic photometric test, Trinder, End Point.

PRINCIPLE :

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

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

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHO}} \text{Cholesterol-4-en-3-one} + H_2O_2$$

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

REAGENTS :

COMPONENTS AND CONCENTRATIONS:

Pipes buffer	:	100 mmol/l
Cholesterol Oxidase	:	> 100 U/l
Peroxidase	:	> 500 U/l
Cholesterol Esterase	:	> 150 U/l
4 - Amino Antipyrine	:	0.5 mmol/l
Phenol	:	10 mmol/l
Preservative & Stabilizer		
Standard	:	200 mg/dl (5.18 mmol/L)

STORAGE INSTRUCTIONS AND REAGENT STABILITY :

The reagent is stable up to the end of the indicated date of expiry on the vial label, if stored at 2 to 8°C, protected from light and contamination is avoided. Do not freeze the reagents!

The standard is stable up to the end of the indicated date of expiry on the vial label, if stored at 2 to 8°C.

Note: It has to be mentioned, that the measurement is not influenced by occasionally occurring color changes, as long as the absorbance of the reagent is < 0.3 at 510 nm.

WARNINGS 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. Take the necessary precautions for the use of laboratory reagents.

WASTE MANAGEMENT :

Please refer to local regulation requirements.

REAGENT PREPARATION :

The reagent and the standard are ready-to-use.

MATERIALS REQUIRED BUT NOT PROVIDED :

NaCl solution 9 g/l, General laboratory equipment, Analyser / Photometer, Pipettes etc.

SPECIMEN :

Serum, heparinized plasma or EDTA plasma from fasting patients.

Stability : 7 days at 4 to 8°C, 3 months at –20°C

Discard contaminated specimens.

ASSAY PROCEDURE :

Application sheets for automated systems are available on request.

Wavelength	:	Hg 510 nm, 546 nm or 505 nm
Optical Path	:	1 cm
Temperature	:	37°C
Mode	:	End Point

Bring all the contents of the kit to Room Temperature prior to use.

Read absorbance of standard, sample against reagent blank.

Label the test tube as blank, standard, sample, control and pipette into respective test tube the reagent, standard, sample, control sample as per the table given below :

	Blank	Standard	Sample / Control
Reagent	1000 µl	1000 µl	1000 µl
Distilled Water	10 µl	—	—
Standard	—	10 µl	—
Sample / Control	—	—	10 µl

Mix and read the absorbance (A) after a 10 minutes incubation but within 30 minutes.

CALCULATION :

With standard or calibrator.

$$\text{Conc. of unknown Sample} = \frac{\text{Concentration of Standard}}{\text{Abs. of Standard} - \text{Abs. of Reagent Blank}} \times \text{Abs. of unknown Sample} - \text{Abs. of Reagent Blank}$$

CONVERSION FACTOR :

Cholesterol [mg/dl] X 0.0259 = Cholesterol [mmol/l]

CALIBRATION :

For the calibration of automated photometric systems use of the commercially available calibrator is recommended.

QUALITY CONTROL :

To ensure adequate quality, use of the commercially available control serum is recommended.

PERFORMANCE CHARACTERISTICS :

MEASURING RANGE :

The test has been developed to determine Cholesterol concentrations within a measuring range from 4 to 700 mg/dl (0.104 to 18.13 mmol/L). When values exceed higher limit of the range, such samples should be diluted 1 + 1 with NaCl solution (9 g/l) and the result multiplied by 2.

SPECIFICITY / INTERFERENCES :

No interference was observed by ascorbic acid up to 5 mg/dl (283.9 µmol/L), bilirubin up to 20 mg/dl (342.00 µmo/L), Hemoglobin up to 0.2 g/dl (2 g/L) and triglycerides up to 600 mg/dl (6.84 mmol/L). A list of drugs and other interfering substances with Cholesterol determination has been reported by Young et al.

SENSITIVITY / LIMIT OF DETECTION :

The lower limit of detection is 4 mg/dl (0.104 mmol/L).

PRECISION :

Intra-assay precision n = 20	Mean [mg/dl]	SD [mg/dl]	CV [%]
Sample 1	234.26	2.29	0.98
Sample 2	196.8	1.67	0.85
Sample 3	188.8	1.31	0.69

Inter-assay precision n = 20	Mean [mg/dl]	SD [mg/dl]	CV [%]
Sample 1	145.8	2.43	1.66
Sample 2	91.89	1.71	1.86
Sample 3	259.9	4.66	1.79

METHOD COMPARISON :

A comparison between Robonik Prietest Cholesterol (y) and a commercially available test (x) using 20 samples gave following results :

Linear Regression : y = 0.9602 x + 7.3582 mg/dl

Correlation Coefficient : r = 0.9762

REFERENCE RANGE :

Desirable	:	≤ 200 mg/dl	(≤ 5.18 mmol/L)
Borderline high risk	:	200 to 240 mg/dl	(5.18 to 6.22 mmol/L)
High risk	:	> 240 mg/dl	(> 6.22 mmol/L)

It is recommended that each laboratory should assign its own reference range.

LITERATURE :

1. Naito, H. K., *Coronary artery disease and disorders of lipid metabolism*, Clinical Chemistry: Theory, Analysis, Correlation, 4th Ed., Kaplan, L.A., Pesce, A.J., Kazmierczak, S. C., (Mosby Inc. eds St Louis USA), (2003).
2. Tietz, N. W., Clinical guide to laboratory tests, 3rd Ed., (W.B. Saunders eds. Philadelphia USA),.
3. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed. AACC Press, 1995.

INSTRUMENT APPLICATION prietest TOUCH	
Name : CHOL	Mod : END-P
Pri.: 510 , Sec.: 0	
Temp: 37C , KF : 1.000	
Vol : 500ul , Unit : mg/dl	
Lag : 5 , Read : NA	
Blk : Y, QC : Y, Norm : Y	
Std : 1 , Concen :	
Std.: 1 = 200	
Normal HI = 200	
Normal LO = 150	
QCNH : *	
QCNL : *	
QCABH = *	
QCABL = *	
Rgnt. Linearity : 700	
NOTE :	
* Indicates user definable parameter.	
NA Implies Not Applicable	

PARAMETERS FOR INSTRUMENT SETTING	
TEST NAME	CHOLESTEROL
Reaction	End Point
Reaction Slope	Increasing
Wavelength 1	510 nm
Temperature	37°C
Zero Setting	Reagent Blank
Standard Conc.	200
Units	mg/dl
Sample Volume	10 µl
Reagent Volume	1000 µl
Incubation Time	10 minutes
Reference Range	150 to 200
Reagent Linearity	700

ROBONIK®

An ISO 9001 : 2008 Certified Company
An ISO 13485 : 2012 Certified Company

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