ROBONIK®

CE

prietest™ ...Clinical Chemistry Reagents

HDL CHOLESTEROL (Precipitant)

In vitro diagnostic test kit, for professional use only

INTENDED USE : Determination of high density lipoprotein cholesterol (HDL-C).
{With use of additional enzymatic cholesterol reagent (Not supplied in a kit)}

ORDERING INFORMATION	**Pack Size**	**Cat No.**
	1 X 50 ml	HDC PPT 0150
	2 X 50 ml	HDC PPT 02 50
	4 X 50 ml	HDC PPT 04 50

CLINICAL SIGNIFICANCE : Cholesterol is a lipidic molecule, insoluble in aqueous medium as plasma, in which it circulates in form of pseudo-emulsion: association of lipids and proteins constituting lipoproteins. These lipoproteins vary quantitatively and qualitatively in their lipidic and proteic composition, inducing structural but such a functional difference to them. The most used classification is that which is based on their difference in density. This explains the name of High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Very Low Density Lipoprotein (VLDL) and the existence of many intermediate fractions which correspond to all the stages of the lipidic metabolism. HDL (lipoproteins of high density) contain approximately 50 % of lipids including 20 % of cholesterol. The HDL molecule plays an integral role in removing cellular cholesterol and thus cellular purification. Many epidemiologic studies confirmed the anti-atherogen function of this fraction leading to the concept of "good cholesterol". Cholesterol HDL represents consequently an element of evaluation of the atherogenesis risk when there is an imbalance of the ratios cholesterol total/cholesterol HDL or cholesterol LDL/cholesterol HDL.

METHOD :
Precipitation. Phosphotungstic acid.

PRINCIPLE :
Chylomicrons, Very Low Density Lipoproteins (VLDL) and Low Density Lipoproteins (LDL) of serum are precipitated by Phosphotungstic acid and Magnesium ions.
After centrifugation, High Density Lipoproteins (HDL) are in the supernatant. Cholesterol included in this phase, is measured by an enzymatic method.

REAGENTS :

COMPONENTS AND CONCENTRATIONS:

Phosphotungustic Acid	:	1.52 gm/l
Magnesium Chloride	:	0.49 gm/l
Standard	:	50 mg/dl (1.3 mmol/L)

STORAGE INSTRUCTIONS AND REAGENT STABILITY :
The reagents are stable up to the end of the indicated date of expiry on the vial label, if stored at 2 to 30°C, protected from light and contamination is avoided. Do not freeze the reagent!

WARNINGS AND PRECAUTIONS :
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 :
Cholesterol CHOD-PAP Reagent, NaCl solution 9 g/l, General laboratory equipment, Analyser / Photometer, Pipettes etc.

SPECIMEN :
Specimen required
Fasting serum.
Conservation and storage
Keep serum at 4°C before analysis.
Sera are stable from 1 to 3 days at 4°C. For a longer storage, freeze at -50°C.

ASSAY PROCEDURE :
1) Sample preparation
Add to 500 µL of sample, 500 µL of precipitating reagent. Mix, wait for 10 minutes and centrifuge at 5 000 r.p.m. for 15 minutes.
The supernatant is collected for HDL determination.

2) HDL determination
The cholesterol kit (to be ordered separately from prietest Cholesterol - 2 X 50, 1X 50) is used for HDL cholesterol determination.
This reagent can be used on most analysers, semi automated analysers and manual method.
The applications are available on request.
Application sheets for automated systems are available on request.

Wavelength	:	Hg 510 nm, 546 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 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
Cholesterol Reagent	1000 µl	1000 µl	1000 µl
Distilled Water	50 µl	—	—
Standard	—	50 µl	—
Sample / Control	—	—	50 µl

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

CALCULATION :
HDL cholesterol:

$$\text{HDL-C [mg / dl]} = \frac{\text{Abs. of Sample} - \text{Abs. of Reagent Blank}}{\text{Abs. of Standard} - \text{Abs. of Reagent Blank}} \times \text{Conc. of HDL Cholesterol Std. [mg /dl]} \times 2$$

LDL CHOLESTEROL :
The following scheme is based on the Friedewald formula which is reliable only if chylomicrons are absent in the sample, the triglycerides concentration is < 400 mg/dl and the samples are not derived from patients with type III hyperlipoproteinemia.

$$\text{LDL - C [mg/dl]} = \text{Total cholesterol} - \text{HDL CHOL} - \frac{\text{Triglycerides}}{5}$$

$$\text{LDL - C [mmol/l]} = \text{Total cholesterol} - \text{HDL CHOL} - \frac{\text{Triglycerides}}{2.2}$$

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 sera is recommended.

PERFORMANCE CHARACTERISTICS :

MEASURING RANGE :
The test has been developed to determine HDL concentrations within a measuring range from 10 to 180 mg/dl (0.26 to 4.66 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 / INTERFERENCE :
No interference was observed by Ascorbic Acid up to 30 mg/dl (1703.4 µmol/L), Bilirubin up to 40 mg/dl (684 µmol/L), Hemoglobin up to 0.4 g/dl (4 g/L) and lipemia up to 500 mg/dl (5.65 mmol/L) Triglycerides. A list of drugs and other interfering substances with HDL determination has been reported by Young et al.

SENSITIVITY / LIMIT OF DETECTION :
The lower limit of detection is 10 mg/dl (0.26 mmol/L).

PRECISION :

Intra-assay precision n = 20	Mean [mg/dl]	SD [mg/dl]	CV [%]
Sample 1	100.1	1.1	1.09
Sample 2	50.6	0.5	0.98
Sample 3	32.8	0.4	1.21

Inter-assay precision n = 20	Mean [mg/dl]	SD [mg/dl]	CV [%]
Sample 1	101.4	0.9	0.88
Sample 2	75.8	0.89	1.17
Sample 3	32.9	0.3	0.91

METHOD COMPARISON :
A comparison between Robonik prietest HDL (y) and a commercially available test (x) using 20 samples gave following results:
Linear Regression : y = 0.9279 x + 0.766 mg/dl
Correlation Coefficient : r = 0.9730

REFERENCE RANGE :

High Risk	:	< 40 mg/dl	(< 1.40 mmol/L)
Low Risk	:	≥ 60 mg/dl	(≥ 1.55 mmol/L)

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

LITERATURE :

1. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), *Executive Summary of the Third Report of the National Cholesterol* Education *Program (NCEP)*, JAMA, (2001), **285**, 2486.
2. Tietz, N.W., Clinical guide to laboratory tests, 3rd Ed., (W.B. Saunders eds. Philadelphia USA), (1995), 334.
3. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed. AACC Press, 1995

INSTRUMENT APPLICATION prietest TOUCH
Name : HDLCHOL, Mod : END-P Pri.: 510 , Sec.: 0 Temp: 37C , KF : 2.00 Vol : 500ul , Unit : mg/dl Lag : 5 , Read : NA Blk : Y, QC : Y, Norm : Y Std : 1 , Concen : Std.: 1 = 50 Normal HI = 60 Normal LO = 40 QCNH : * QCNL : * QCABH = * QCABL = * Rgnt. Linearity : 180 NOTE : * Indicates user definable parameter. NA Implies Not Applicable

PARAMETERS FOR INSTRUMENT SETTING	
TEST NAME	HDL CHOL
Reaction	End Point
Reaction Slope	Increasing
Wavelength 1	510 nm
Temperature	37°C
Zero Setting	Reagent Blank
Standard Conc.	50
Units	mg/dl
Sample Volume	50 µl
Reagent Volume	1000 µl
Incubation Time	10 minutes
Reference Range	40 to 60
Reagent Linearity	180

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An ISO 13485 : 2012 Certified Company

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