Pariksha Biotech
A game changer in IVD

Cholesterol Estimation Kit

High-Q Cholesterol - ML

(CHOD/PAP Trinder's Method)

HIGH®
Command on Quality

Intended Use:
Kit for the quantitative determination of cholesterol in human serum and plasma.

Summary and Clinical Significance:
Cholesterol is a steroid with a secondary hydroxyl group in the C3 position. It is synthesized in many types of tissue, but particularly in the liver and intestinal wall. Approximately three quarters of cholesterol is newly synthesized and a quarter originates from dietary intake. Cholesterol assays are used for screening for atherosclerotic risk and in the diagnosis and treatment of disorders involving elevated cholesterol levels as well as lipid and lipoprotein metabolic disorders. Cholesterol analysis was first reported by Liebermann in 1885 followed by Burchard in 1889. In the Liebermann-Burchard reaction, cholesterol forms a blue-green dye from polymeric unsaturated carbohydrates in an acetic acid/acetic anhydride/concentrated sulfuric acid medium. The Abell and Kendall method is specific for cholesterol, but is technically complex and requires the use of corrosive reagents. In 1974, Roeschlau and Allain described the first fully enzymatic method. This method is based on the determination of Δ4 cholestenone after enzymatic cleavage of the cholesterol ester by cholesterol esterase conversion of cholesterol by cholesterol oxidase, and subsequent measurement by the Trinder reaction of the hydrogen peroxide formed. Optimization of ester cleavage (>99.5%) allows standardization using primary and secondary standards and a direct comparison with the CDC and NIST reference methods. The High-Q Cholesterol-ML assay meets the 1992 National Institutes of Health (NIH) goal of less than or equal to 3% for both precision and bias.

Principle:

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

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{Cholest-4 ene 3-one} + H_2O_2$$

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

Normal Range:
Total Cholesterol: 130 - 250 mg/dl
It is recommended that laboratories establish their own normal range.

Reagent Components:

Cholesterol Oxidase	- 1 KU/L
Cholesterol Esterase	- 07 KU/L
Peroxidase	- 5 KU/L
4-Aminoantipyrine	- 0.5 m mol/L
Phenol	- 20 m mol/L
Phosphate Buffer	- 50 m mol/L. pH-7.0
Triton X 100	- 0.1%
Activators & Stabilizers.	

Storage and Stability:
High-Q Cholesterol-ML is available as Single Liquid Reagent and is ready to use. Reagent and Standard should be stored at 2-8ºC and are stable till the expiry date mentioned on the labels.

Specimen:
Serum / Heparinised or EDTA Plasma.

Procedure:

Pipette into 3 test tubes labeled Blank (B), Standard(S) and Test (T) as shown below:

Reagent	B	S	T
Cholesterol Reagent	1.0 ml	1.0 ml	1.0 ml
Cholesterol Standard (Conc. 200 mg/dl)	--	10 µl	–
Specimen	--	--	10 µl

Mix well and incubate for 5 minutes at 37°C or 10 minutes at R.T. Read the absorbance of Standard (S), Test (T) against Reagent Blank (B) at 505 nm (490-550 nm).

Calculations:

$$\text{a) Total Cholesterol (in mg/dl)} = \frac{\text{Abs. of Test}}{\text{Abs. of Standard}} \times 200$$

System Parameters:

Reaction Mode	:	End Point
Units	:	mg/dl
Wave Length	:	505 nm (490-550)
Blanking with	:	Reagent
Flow Cell Temp.	:	37°C
Low Normal	:	130
High Normal	:	250
Sample Volume	:	10 µl
Reagent Volume	:	1000 µl
Linearity	:	1000
Standard Conc.	:	200

Quality Control:
To ensure adequate quality control, the use of commercial reference control serum is recommended with each assay batch. Use of Quality Control material checks both, the instrument and the reagent functions.

Notes:

1) The Enzyme Reagent on storage at 2-8°C develops a slight pink color. However, this does not affect the performance of the test.
2) As with all the diagnostic procedures, the physician should evaluate data obtained by the use of this kit in light of other clinical information.

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Limitations - interference:

No significant interference was observed from Bilirubin up to 25 mg/dl,Hemoglobin up to 500 mg/dl and Triglycerides up to 1250 mg/dl.

Measuring/reportable range:

3-1000 mg/dl

Determine samples having higher activities via the rerun function. On instruments without rerun function, manually dilute the samples with 0.9% NaCl-solution or distilled/deionized water (e.g. 1 + 2). Multiply the result by the appropriate dilution factor (e.g. factor 3).

Analytical sensitivity (lower detection limit)

Detection limit : 3 mg/dl

The lower detection limit represents the lowest measurable cholesterol concentration that can be distinguished from zero.

Imprecision:

Reproducibility was determined using controls. The following results were obtained:

within run

Sample	Mean mg/dl	SD mg/dl	% CV
Control serum 1	103	1.35	1.32
Control serum 2	158	0.98	0.62
Control serum 3	180	0.99	0.55

Between day

Sample	Mean mg/dl	SD mg/dl	% CV
Control serum1	124	1.71	1.38
Control serum 2	162	3.13	1.93
Control serum 3	186	1.97	1.06

Method comparison:

A comparison of the High-Q Cholesterol-ML (y) with a commercial obtainable assay (x) gave following result:

y = 1.006 x + 0.258 ; r = 0.999

References:

1) Allain, C.C. Clin. Chem 20, 470 (1974)
2) Abell L. et al. Standard Methods in Clinical Chemistry 1958; 26:2.
3) Allain C.C. et al. Clin Chem 1974;20:470
4) Bablok W. et al. A General Regression Procedure for Method Transformation. J Clin Chem Clin Biochem 1988;26:783-790

Ordering information

Ref./Cat.	Pack Size	Presentation
P-CHO - 50	2 x 25 ml	Mono Reagent
P-CHO-100	4 x 25 ml	
P-CHO-250	5 x 50 ml	
P-CHO - 500	10 x 50 ml	

Product Features

- Liquid stable, ready to use mono reagent
- One step End Point assay.
- Aqueous Cholesterol standard provided (Standard Conc: 200 mg/dl)
- Cholesterol Linearity: 1000 mg/dl
- Measuring Wavelength 505 nm (490 – 550 nm)
- Serum / Heparinized or EDTA Plasma as Specimens
- Available as multipurpose reagents and dedicated system packs

Symbols used with IVD devices

	Date of manufacture		Manufactured by
IVD	In vitro diagnostic device		Keep away from sunlight
	Do not freeze		This way up
	Use by (yyyy-mm-dd or mm/yyyy)	R	Reagent
CAL	Calibrator Material	LOT	Batch code
2°C 8°C	Temperature limitation (store at)	CONTROL	Control
	Consult instructions for use		Keep dry Keep away from rain
REF	Catalog Number		

eIFU Indicator

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SCAN TO EXPLORE MORE

Manufactured in India by :
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AN ISO 13485 Certified Company

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