

CHOLESTEROL (Liquid)

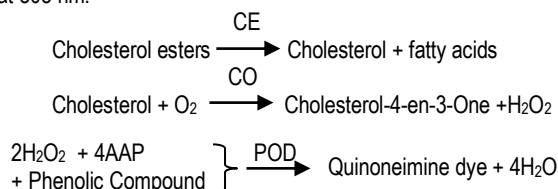
ENZOPAK

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Liquid Reagent for quantitative estimation of cholesterol in serum or plasma.

PRINCIPLE:

The cholesterol esters are hydrolysed to free cholesterol by cholesterol esterase (CE). The free cholesterol is then oxidised by cholesterol oxidase (CO) to cholesterol 4-en-3-one with the simultaneous production of hydrogen peroxide. The hydrogen peroxide reacts with 4-aminoantipyrine and phenolic compound in the presence of peroxidase to yield a coloured complex which is read at 505 nm.



The intensity of colour produced is directly proportional to the concentration of total cholesterol in the sample.

REAGENT COMPOSITION

Active Ingredients	Concentration
Reagent-1	
• Cholesterol oxidase	≥ 500 U/L
• Cholesterol Esterase	≥ 600 U/L
• Peroxidase	≥ 6000 U/L
• 4-Amino Antipyrine	0.5 mmol/L
• Phenolic compound	20 mmol/L
• Surfactant	10 mmol/L
• Buffer	100 mmol/L

pH 7.00± 0.1 at 25°C

Cholesterol Standard (200 mg/dl)

Also contains non-reactive fillers and Stabilizers.

PRESENTATION :

	No. of bottles		
	2x50ml	4x50ml	5x100ml
CHOLESTEROL-L (Ready to use)	2	4	5
CHOLESTEROL-STANDARD (200 mg/dl)	1	1	1

The reagent blank of cholesterol -L can be controlled if stored frozen. However avoid repeated freezing & thawing. Alternatively all reagents may be stored at 2-8°C.

PRECAUTIONS :

CHOLESTEROL -L is for *in-vitro* diagnostic use.

Reagent Contains Sodium Azide, DO NOT INGEST.

PREPARATION OF WORKING REAGENT :

ENZOPAK CHOLESTEROL-L is Ready-to-use. No Reconstitution is required.

REAGENT STORAGE AND STABILITY :

ENZOPAK CHOLESTEROL-L reagents are stable at 2-8°C until the expiry date stated on the label. However refer presentation.

SPECIMEN COLLECTION :

Fresh, clear serum with no hemolysis under fasting condition is specimen of choice. Plasma collected with Heparin or EDTA as anticoagulants can also be used.

NOTE :

A special surfactant, Lipid Clearing Factor (L.C.F.) is added to the Reagent to solubilise the lipemic sera (causing turbidity or opalescence) which adds to the accuracy of results.

PROCEDURE :

REACTION PARAMETERS :

- Type of Reaction : End Point
- Wavelength : 505 nm (505-530 nm)
- Flowcell Temperature : 30 °C / 37 °C
- Sample Volume : 10 µl
- Reagent Volume : 1.0 ml.
- Incubation time : 30 min. R.T. / 10 min. 37° C
- Std. Concentration : 200 mg/dl
- Light Path : 1.0 cm.
- Zero setting with : Reagent Blank

PIPETTE IN TO TUBES	BLANK	STD	TEST
CHOLESTEROL REAGENT (ml.)	1.0	1.0	1.0
STANDARD (ml)	-	0.01	-
SAMPLE (ml)	-	-	0.01

Mix well and incubate for 10 minutes at 37°C or 30 minutes at R.T. (25 ± 5°C) Read absorbance of standard and test at 505 nm (505-530) (Green filter) against reagent blank.

TEST RESULTS:

$$\text{Cholesterol Concentration (mg/dL)} = \frac{\text{Absorbance of test}}{\text{Absorbance of Std.}} \times 200$$

LIMITATIONS FOR INTERFERENCE:

As per studies carried out for interference. Following results were obtained.

- No Interference from Hemoglobin upto 375 mg/dl.
- No Interference from free Bilirubin upto 7.5 mg/dl.
- No Interference from Lipemic (Measured as Triglycerides) upto 1000 mg/dl.

NORMAL VALUES :

Serum Cholesterol : 140 to 250 mg/dl.

LINEARITY :

This procedure is linear upto 1000 mg/dl. For sample values higher than 1000 mg/dl, dilute the sample suitably with 0.9 % saline and repeat the assay. Apply dilution factor to obtain test results.

REFERENCES :

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- Rochalu, B.Bernt, E. Gruber, W., Enzymatische Bestimmung des Gesamt-Cholesterins in serum, Z. Klin. Chem. Klin Biochem 12,226 (1974)
- Pescse, M.A. Bodourian, S.H. Interference with the enzymatic measurement of cholesterol in serum by use of five reagent kits. Clin. Chem. 23,757 (1977).

