CHOLESTEROL-L (Single Liquid)

ENZOPAK

(CHOD-PAP, Enzymatic)

Last update 09-2020

Ref. CC3-CLE.05M, 2x60 ml
CC3-CLE.5MU, 5x60 ml
CC3-CLE.5MV, 5x120 ml

INTENDED USE

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 cholesten 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.

$$\text{Cholesterol esters} \xrightarrow{CE} \text{Cholesterol + fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{CO} \text{Cholesterol-4-en-3-One} + H_2O_2$$

$$2H_2O_2 + 4AAP + \text{Phenolic Compound} \xrightarrow{POD} \text{Quinoneimine dye} + 4H_2O$$

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

PRESENTATION

All reagents to be stored at 2-8^{0}C	No. of bottles		
	2x60ml	5x60ml	5x120ml
• Cholesterol-L (Ready to use)	2	5	5
• Cholesterol-Standard (200 mg/dl)	1	1	1

FINAL REAGENT COMPOSITION

Active Ingredients	Concentration
• 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^{0}C

Cholesterol Standard (200 mg/dl)

Also contains non-reactive fillers and Stabilizers.

PRECAUTION

Cholesterol -L is for *in-vitro* diagnostic use.
Reagent Contains Sodium Azide, DO NOT INGEST.

PREPARATION OF WORKING REAGENT

Cholesterol-L reagent is Ready-to-use.

REAGENT STORAGE AND STABILITY

Cholesterol-L reagents are stable at 2-8^{0}C until the expiry date stated on the label.

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.

REACTION PARAMETERS

- Type of Reaction : End Point
- Wavelength : 505 nm (505-530 nm)
- Flowcell Temperature : 30 ^{0}C / 37 ^{0}C
- Sample Volume : 10 µl
- Reagent Volume : 1.0 ml.
- Incubation time : 30 min. R.T. / 10 min. 37^0 C
- Std. Concentration : 200 mg/dl
- Light Path : 1.0 cm.
- Zero setting with : Reagent Blank

TEST PROCEDURE

Pipette In To Tubes	BLANK	STANDARD	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^{0}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

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.

NOTE

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

REFERENCES

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

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