



CHOLESTEROL

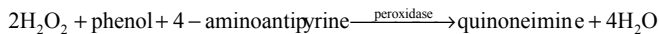
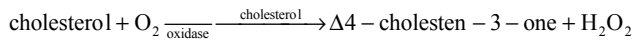
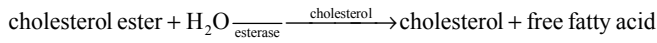
Enzymatic colorimetric test
(CHOD-PAP)/ liquid

Cat.No. 101-0440

Size: 4x250 ml

PRINCIPLE:

Cholesterol esterase catalyzes the hydrolysis of cholesterol esters into free cholesterol and fatty acids. The free cholesterol is then oxidized to 4-cholesten-3-one and hydrogen peroxide in the presence of cholesterol oxidase. Phenol and 4-aminoantipyrine then combine with the hydrogen peroxide in the presence of peroxidase to produce red quinoneimine. The intensity of the color thus produced is directly proportional to the total cholesterol concentration of the sample.



SAMPLE:

Serum or EDTA - plasma.

Cholesterol in serum is stable for 7 days at +2 °C to +8 °C and three months frozen (-20 °C).

REAGENTS:

- Buffer

Pipes buffer, pH 6.9	90 mmol/L
Phenol	26 mmol/L
Peroxidase	1250 U/L
Cholesterolesterase	1000 U/L
Cholesteroxidase	300 U/L
4-aminoantipyrine	0.4 mmol/L
- Cholesterol standard
Standard concentration see on the vial label

Store at +2 °C to +8 °C

PREPARATION OF REAGENTS

Liquid reagent, ready to use.

This reagent is stable up to the date of expiration at +2 °C to +8 °C. Avoid direct sunlight.

PROCEDURE:

Wavelength:	505 nm (500 - 550 nm) or Hg 546 nm
Colour stability:	60 min.
Cuvette:	1 cm light path
Temperature:	room temperature or 37 °C
Zero:	reagent blank

Pipette into test tubes:	Reagent blank	Standard	Sample
Standard	-	10 µl	-
Sample	-	-	10 µl
Working reagent	1000 µl	1000 µl	1000 µl

Mix, incubate 5 min. at 37°C or 10 min. at 15 – 25 °C. Measure absorbance of sample and of standard against the reagent blank within 60 min.

CALCULATION:

$$\frac{A_{\text{sample}}}{A_{\text{standard}}} \times \text{stand. conc.} = \text{Cholesterol conc.}$$

EXPECTED VALUES:

Normal values:	up to 5.7 mmol/L (220 mg/dL)
Suspect:	5.7 - 6.7 mmol/L (220 - 260 mg/dL)
Elevated:	> 6.7 mmol/L (260 mg/dL)

LINEARITY:

up to 15.4 mmol/L (600 mg/dL)

QUALITY CONTROL:

CONTRO-N	20 x 5 ml	Cat. No. 101-0083
CONTRO-P	20 x 5 ml	Cat. No. 101-0084

NOTE:

- Sample with cholesterol concentration > 15.4 mmol/L has to be diluted 1:2 with physiological solution (result x 2).
- Haemoglobin up to 2 g/L, bilirubin up to 85 µmol/L, triglycerides up to 11 mmol/L and proteins up to 100 g/L do not interfere with test.
- Ascorbic acid's concentration ≥ 50 mg/L, methyl dopa ≥ 50 mg/L, 4-methylaminoantipyrine ≥ 120 mg/L decrease results.
- Working solution must not get in touch with skin and mucous membranes (the reagents contain phenol).

REFERENCES:

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- Richmond, W., Clin. Chem. 19, 1350 - 1356 (1973).
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- Deeg R. and Ziegenohm, J. Clin Chem 28, 1574 (1982)