H-1117 Budapest,
Budafoki street 111-113
Tel.: +36-1-205-3617
Tel./Fax: +36-1-205-3616

CHOLESTEROL PAP

CONCENTRATED LIQUID REAGENT

CE

Order information:

Catalogue number	Size
9801C	10 x 65 ml
9802C	4 x 65 ml

Reagent kit for quantitative in vitro determination of cholesterol in serum and plasma.

Summary

The biosynthesis of cholesterol predominantly takes place in the liver and in intestinal mucosa, but almost all cells synthesize it. It is a constituent of many membranes, it is also essential in the synthesis of bile acids and steroid hormones. It circulates in blood as cholesterol ester bound to beta lipoproteins. The measurement of the level of cholesterol as well as triglycerides and lipoproteins is important in examining the metabolism of lipids. Changes in the level of cholesterol mainly reflect disorders of liver function. Cholesterol level is increased in obstructive jaundice, diabetes mellitus and hypothyroidism. The level is decreased in some cases of hyperthyroidism and certain forms of anaemia. Identification of the different density fractions (HDL, LDL, VLDL) as well as total Cholesterol plays a role in the diagnosis.

Method

Cholesterol oxidase/PAP method

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{cholesterol esterase}} \text{cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \text{4-cholesten-3-on} + H_2O_2$$

$$H_2O_2 + \text{phenol} + \text{4-aminoantipyrine} \xrightarrow{\text{peroxidase}} \text{red quinone dye} + 2H_2O$$

Reagents

Composition and concentrations

PIPES buffer pH=6,7	50 mmol/l
Phenol	100 mmol/l
Cholesterol oxidase	1000 U/l
Cholesterol esterase	1000 U/l
Peroxidase	5000 U/l
4-aminoantipyrine	2,5 mmol/l
Sodium-cholate	0,5 mmol/l

Storage and stability

The reagent is stable up to the end of the indicated month of expiry without opening, if stored at 2 – 8°C, protected from light and contamination is avoided. Do not freeze!
Onboard stability after opening and the frequeny of calibration is 60 days.

Warnings and precautions

Do not use reagents after the expiry date stated on each reagent container label.

Chemical safety

This product is not classified as dangerous. Safety data sheet is available upon request. The product contains sodium azide. Sodium azide can react with copper and lead plumbing to form explosive metal azides. If disposal into a drain is in compliance with federal, state, and local requirements, flush reagents with a large amount of water to prevent the buildup of azides.

Preparation

The reagent is ready for use.

Sample

Serum, heparin, citrate or EDTA plasma.

Stability in serum:	7 days at 4 – 8°C
	7 days at 20 – 25°C
	3 month at -20°

Expected values and reference range

Serum 2,90 – 5,21 mmol/l

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

Assay procedure

Wavelength:	505 nm / 700 nm (primary/secondary)
Optical path:	1 cm
Temperature:	37°C
Measurement:	against reagent blank
Reaction:	endpoint, increasing

	blank	sample or standard
reagent	200 µl	200 µl
dist. water (diluent)	800 µl	800 µl
dist. water (blank)	10 µl	-
sample or standard	-	10 µl

Mix and incubate for 5 minutes and read the absorbance against reagent blank

Calculation

Cholesterol[mmol/l]=ΔA sample/ΔA standard × standard concentration[mmol/l]

Conversion factor

[mmol/l]×38,64=[mg/dl]

Calibration and quality control

S1: Distilled water
S2: Cholesterol standard Cat.: 50611N or
Roche C.F.A.S. (Calibrator for automated system) or
Randox Calibration Serum Level I or
Randox Calibration Serum Level II
Calibration is recommended:

- after opening new reagent batch
- after system maitenance or troubleshooting

For internal quality control, two levels controls are recommended (normal and pathological) at least once a day. The measured values must in the range which was given by the control's manufacturer. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Preformance characteristics

Measuring range

The method is linear in the range 0,12 – 11,3 mmol/l

Interferences

No significant intereference was observed by bilirubin up to 1000 µmol/l bilirubin, triglycerides up to 4 mmol/l, hemoglobin up to 0,8 g/l and ascorbate up to 0,075 g/l. Significant interference: >10%.

Limit of detection

The limit of detection is 0,012 mmol/l

Precision

Repeatability	mean	SD	CV
n = 20	[mmol/l]	[mmol/l]	[%]
normal sample	4,1	0,03	0,86
pathological sample	7,4	0,04	0,54
Reproduceability	**mean**	**SD**	**CV**
n = 10	[mmol/l]	[mmol/l]	[%]
normal sample	4,02	0,08	1,89
pathological sample	7,25	0,06	0,87

Method comparison

Comparison with the non-concentrated reagent.
analyser: Advia 1600
number of samples: 181
range: 1,33 – 10,65 mmol/l
correlation coefficient: 0,9983
regression line equation: y = 0,95x + 0,277
(x= normal reagent, y= concentrated reagent)

For in vitro diagnostic use only!

The following symbols can be used on the labels

IVD — In vitro diagnostic device

Manufacturer

CE — CE-marking

Temperature limitations

Use by (year/month)

LOT — Batch code

REF — Catalogue number

This way up

Biological risk

Literature

Allain C.C and al., Clin.Chem.,20, (1974), 470.
Tietz Clinical Guide To Laboratory Tests, 4th edition, Elsevier, 2006