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CHOLESTEROL ADPS

CE

STABLE LIQUID REAGENT

Cat. No.:	46061,46061S	46062,46062S	46063
	120 ml	600 ml	10x30 ml
	(1x80 ml+1x40 ml)	(1x400 ml+1x200 ml)	(10x20 ml+ 10x10 ml)

Reagent kit for the determination of total cholesterol concentration in serum. Enzymatic colorimetric method (ADPS).

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.

Principle

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Cholesterol-esterase}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol-oxidase}} \text{4-Cholesten-3-one} + H_2O_2$$

$$2H_2O_2 + \text{ADPS} + \text{4-Aminoantipyrine} \xrightarrow{\text{peroxidase}} \text{purple quinone} + 4H_2O$$

ADPS=N-Ethyl-N-sulfopropyl-anisidine

Reference values

Serum cholesterol: **2.8-5.2 mmol/l (109-202 mg/dl)**

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

Reagents

1. Reagent (R1)

Pipes buffer, pH=7.00	50 mmol/l
ADPS	1 mmol/l
Cholesterol esterase	180 U/l
Peroxidase	1000 U/l
Ascorbate oxidase	≥3000 U/l

2.Reagent (R2)

4-Aminoantipyrine	0.9 mmol/l
Cholesterol oxidase	200 U/l

3. Cholesterol standard

Ready for use.For details please check the insert.
Available only in Cat. No.: 46061S and 46062S

Precaution

Discard cloudy reagent. Avoid contamination by using clean laboratory materials (pipettes, plastic vials for analyzers).
The reagents contain 0.1 % sodium azide. To avoid the possible build-up of azide compounds, flush waste-pipes with water after the disposal of undiluted reagent.

Samples

Serum free of haemolysis.

PROCEDURE

Preparation and stability of working reagent

•One-reagent procedure:
Mix 2 volumes of R1 with 1 volume of R2.

Stability: at 20-25°C: 2 weeks
at 2-8°C : 1 month

•Two-reagent procedure:
The reagents are ready for use.
If the absorbance of working reagent is higher than 0.1 at 546 nm the reagent can not be used.

Assay conditions

Wavelength: 546 (520-570) nm
Temperature: 37°C
Cuvette: 1 cm light path
Read against: reagent blank
Method: endpoint (increasing)

One-reagent procedure

	Blank	Standard	Sample
Working reagent	1 ml	1 ml	1 ml
Distilled water	10µl		
Standard		10µl	
Sample			10µl

Mix and read the absorbance (A) after a 5-minute incubation.

Two-reagent procedure

	Blank	Standard	Sample
R1	1 ml	1 ml	1 ml
Distilled water	15µl		
Standard		15µl	
Sample			15µl

Mix and wait 1 minute and add:

R2	500µl	500µl	500µl

Mix and read the absorbance (A) after a 5-minute incubation.

Calibration (37°C, Cholesterol-oxidase method)
S1: Distilled water
S2: Cholesterol standard Cat. No.: 50611N or
Roche C.F.A.S. (Calibrator for automated system)
Randox Calibration Serum Level I or
Randox Calibration Serum Level II

Calibration frequency

Calibration is recommended:
- after reagent lot change,
- as required following quality control procedures.

Calculation using calibration

$$\frac{A_{sample}}{A_{standard}} xC_{standard} = C_{sample}$$

A = Absorbance, C = Concentration

Quality control

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

PERFORMANCES DATA

The following data were obtained using the Hitachi 717 analyzer.

Linearity

The test is linear up to 15.5 mmol/l (600 mg/dl).

Sensitivity

It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used. Under manual conditions however, a change of 0.001 Abs is equivalent to 0.009 mmol/l (0,35 mg/dl) cholesterol concentration at 546 nm.

Precision

	Reproducibility		
	Average concentration (mmol/l)	SD	CV%
Sample I	2.73	0.05	1.83
Sample II	4.99	0.20	4.01

Correlation

Comparative studies were done to compare our reagent with another commercial Cholesterol reagent.
The results from these studies are detailed below.
Correlation coefficient: r=0.9960
Linear regression: y (mmol/l)= 0.980x+0.107
(x= other commercial reagent, y= own reagent).

Specificity

Bilirubin 855 µmol/l (50mg/dl), lipid 250mg/dl, glucose 55.5 mmol/l (1000mg/dl) and ascorbic acid 1.42 mmol/l (25mg/dl) don't interfere with the assay up to the given levels.

NOTE

Do not use reagents after the expiry date stated on each reagent container label. Do not use products, test solutions and reagents described above for any purpose other than described herein.

For *in vitro* diagnostic use only.

The following symbols are used on labels

IVD For in vitro diagnostic use

Use by (last day of the month)

Temperature limitation

LOT Batch Code

REF Code

Bibliography

Allain C.C and al., Clin. Chem., 1974;20: 470.