BIOLABO
www.biolabo.fr

MANUFACTURER:
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CHOLESTEROL CHOD PAP

Reagent for quantitative determination of Total Cholesterol in human serum and plasma.

REF 80106	**R1**	2 x 100 mL	**R2**	2 x 100 mL	**R3**	1 x 5 mL
REF 87356	**R1**	10 x 100 mL	**R2**	10 x 100 mL	**R3**	1 x 5 mL

TECHNICAL SUPPORT AND ORDERS
Tel: (33) 03 23 25 15 50
support@biolabo.fr
Latest revision: www.biolabo.fr

CE

IVD

Made In France

I: corresponds to significant modifications

INTENDED USE

This reagent is designated for professional use in laboratory. (manual or automated procedure).
It allows quantitative determination of Total Cholesterol in human serum and plasma. To be used as part of assessment of lipids homeostasis.

GENERALITIES (1) (2)

Hypercholesterolemia can be observed in case of dietary imbalance, in in hepatic and thyroid disorders, certain cases of diabetes, nephrotic syndrome, pancreatitis, myeloma or familial hypercholesterolemia.
Total cholesterol increased levels may be isolated or associated to other increased lipids (hyperlipidemia).
A decreased level of cholesterol may be due to deficiencies or malnutrition, cancer or hyperthyroidism.

PRINCIPLE (4)

Enzymatic method described by Allain and al., which reaction scheme is as follows:

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

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

2 H_2O_2 + Phenol + PAP $\xrightarrow{\text{POD}}$ Quinoneimine (pink) + 4 H_2O

REAGENTS

R1 CHOLESTEROL CHOD PAP Buffer

Phosphate buffer	100	mmol/L
Chloro-4-phenol	5	mmol/L
Sodium Cholate	2.3	mmol/L
Preservative		

According to 1272/2008 regulation, this reagent is not classified as dangerous

R2 CHOLESTEROL CHOD PAP Enzymes

Cholesterol oxidase (CO)	≥ 100	IU/L
Cholesterol esterase (CE)	≥ 170	IU/L
Peroxidase (POD)	≥ 1200	IU/L
4 - Amino – antipyrine (PAP)	0.25	mmol/L
PEG 6000	167	µmol/L

According to 1272/2008 regulation, this reagent is not classified as dangerous

R3 CHOLESTEROL CHOD PAP Standard

Cholesterol 200 mg/dL (5.17 mmol/L)

Attention Danger

Skin Irrit. 2 : H315 – Causes skin irritation
Eye Dam. 1: H318 – Causes serious eye damage
Flam. Liq. 3: H226 – Flammable liquid and vapor

P210: Keep away from heat/sparks/open flames/hot surfaces – No smoking,
P280: Wear protective gloves/protective clothing/eye protection/face protection,
P302+P352: IF ON SKIN: Wash with soap and water,

P305+P351+P338: IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing,
P501: Dispose of contents/container in accordance with dangerous waste regulations.
I Classification due to N-Propanol and Tergitol 10 - < 25% For more details, refer to Safety Data Sheet (MSDS)

SAFETY CAUTIONS

- Refer to current Material Safety Data Sheet available on request or on www.biolabo.fr
- Verify the integrity of the contents before use.
- Waste disposal: Respect legislation in force in the country.
- All specimens or reagents of biological origin should be handled as potentially infectious. Respect legislation in force in the country.

Any serious incident that has occurred in connection with the device is notified to the manufacturer and the competent authority of the Member State in which the user and/or patient is based.

REAGENTS PREPARATION

Use a non-sharp instrument to remove aluminum cap.
Add promptly the content of vial R2 into vial R1.
Mix gently until complete dissolution.
Vial R3: Ready to use

STABILITY AND STORAGE

Stored away from light, well cap in the original vial at 2-8°C, reagent is stable when stored and used as described in the insert:

Unopened,
- Until the expiry date stated on the label of the Kit.

Once opened:
- Reconstitute immediately substrate (vial R2)
- Standard (vial R3): Transfer the requested quantity, recap and store at 2-8°C.

Once reconstituted:
- Transfer requested quantity and store in the original vial at 2-8°C.
- Working reagent is stable at least for 2 years.
- Discard any reagent if cloudy or if reagent blank at 500 nm > 0.400.
- Don't use working reagent after expiry date.

SPECIMEN COLLECTION AND HANDLING (2)

Serum or plasma (Heparin or EDTA). Do not use oxalate, fluoride or citrate. Collect on fasting patient. Separate serum from cells within 2 h.

Cholesterol is stable in the specimen for:
- 5-7 days at 2-8°C
- 3 months at –20°C
- Many years at –70°C.
- Avoid repeated freezing and thawing

LIMITATIONS (2) (3) (5)

Enzymatic methods increase analytic specificity. CO also reacts with 3β-hydroxycholesterols (insignificant quantity in human serum – i.e. DHEA, pregnenolone).

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S or N. W. Tietz.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Spectrophotometer or Biochemistry Clinical Analyzer

QUALITY CONTROL

- REF 95010 EXATROL-N Level I.
- REF 95011 EXATROL-P Level II.
- External quality control program.

It is recommended to control in the following cases:

- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:

1. Prepare a fresh control serum and repeat the test.
2. If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test.
3. If control is still out of range, repeat with a new vial of reagent.

If control is still out of range, please contact BIOLABO technical support or your local Agent.

REFERENCE INTERVAL (2)

Values for adults, in term of risk for atherosclerotic diseases:

Total cholesterol	mg/dL	[mmol/L]
Recommended values	< 200	[< 5.18]
Low risk	200-239	[5.18-6.19]
High risk	≥ 240	[≥ 6.22]

Each laboratory should establish its own normal ranges for the population that it serves.

PERFORMANCES

Linearity range: between 50 mg/dL and 500 mg/dL

Detection limit: approximately 1 mg/dL (0.026 mmol/L)

Precision on Kenza analyzer:

Within-run N = 20	*Normal level*	*Medium level*	*High level*
Mean (mg/dL)	88	180	270
S.D. (mg/dL)	2	4	6
C.V. %	2.3	2.4	2.1

Between run N = 20	*Normal level*	*Medium level*	*High level*
Mean (mg/dL)	88	178	303
S.D. (mg/dL)	3	5	7
C.V. %	3.6	3.1	2.4

Comparison study with commercially available reagent:

y = 0.957 x + 6.4 r = 0.9904

I Analytical Sensitivity: approx.0.235 abs for 100 mg/dL (Manual method, 500nm, 1cm pathlength)

Interferences:

Total bilirubin	Negative interference from 250 µmol/L
Ascorbic acid	Negative interference from 8 mg/dL
Glucose	No interference up to 1264 mg/dL
Haemoglobin	No interference up to 310 µmol/L

Other substances may interfere (see § Limitations)

CALIBRATION (6)

- REF 95015 Multicalibrator (traceable to SRM1951c)

or

- Standard (vial R3)

The calibration frequency depends on proper instrument functions and on the preservation of the reagent.

Make a new calibration when changing reagent batch, if quality control results are found out of the range and after maintenance operations.

PROCEDURE

Manual method:

Let stand reagent and specimens at room temperature.

Reagent	1000 µL
Blank, Standard, Control or specimen	10 µL

Mix. Let stand for 10 minutes at room temperature or 5 minutes at 37°C.
Record absorbances at 500 nm (480-520) against reagent blank.
Color is stable for 1 hour.

1- Performances with manual procedure should be validated by user.

2- Kenza applications and other applications proposal are available on request.

CALCULATION

Manual Procedure:

$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times \text{Standard concentration}$$

Automatic Biochemistry analyzer:

The analyzer provides directly final result.
For more details about calibration and calculation of results, refer to User's manual and specific application.

REFERENCES

(1) TIETZ N.W. Textbook of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 826-835.

(2) Clinical Guide to Laboratory Test, 4th Ed., N.W. Tietz (2006) p. 244-249.

(3) YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p. 3-143 to 3-164

(4) Allain C. C. et al., Clin. Chem. (1974), 20/4, p.470-475

(5) Allan C., Deacon et Peter J. G. Dawson, Clin. Chem. (1979) 25/6, p.976-984

(6) SRM : Standard Reference Material ®

Manufacturer	Expiry date	In vitro diagnostic	Storage temperature	Dematerialized water	Biological risk
Product Reference	See Insert	Batch number	Store away from light	Sufficient for	Dilute with