



CHOLESTEROL TOTAL - L



Enzymatic colorimetric method for the quantitative determination of Total Cholesterol in serum or plasma



ORDER INFORMATION

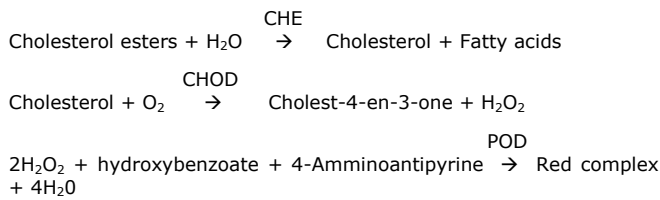
REF	Kit size
GA4340 00	12x50 ml
KL4340 00	12x60 ml
BK4340 00	4x60 ml

INDICATION

Cholesterol determination is used for the diagnosis and monitoring of lipidic metabolism diseases.

METHOD PRINCIPLE

The measurement is based on the following enzymatic reactions:



The intensity of the red complex is proportional to the total cholesterol present in the sample.

COMPOSITION

REAGENT A:

Good buffer, pH 6.7	50 mmol/l
Cholesterol oxidase (CHOD)	≥ 100 U/l
Cholesterol esterase (CHE)	≥ 300 U/l
Hydroxybenzoic acid	12 mmol/l
4-Aminoantipyrine	0.3 mmol/l
Peroxidase (POD)	≥ 500 U/l
Sodio azide	≤ 0.095 g/l

STANDARD:

Cholesterol	1x5 ml
Verified against NIST reference material.	200 mg/dl

Irritant (x_i) **R41; S7-16-24-26-39**

Preparation

Reagents are liquids ready to use.

Storage and stability

Store at 2-8 °C. Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label if contamination and evaporation are avoided, protected from light.

The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

A slight pink colouring of Reagent A does not interfere with the results. Reagent A has to be limpid, eliminate turbidity, if present.

ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l

SAMPLES

Serum, heparin or EDTA plasma. Do not use fluoride, citrate and oxalate as anticoagulant.

Separate serum from cells as soon as possible.

Stable 6 days at 2-8 °C

Specimen collection / Preanalytical factors

It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

INTERNAL QUALITY CONTROL

It is recommended to use controls with known total cholesterol concentration. Check that the values obtained are within the reference range provided.

ANALYTICAL PROCEDURE

Allow the reagents to reach working temperature before using.

Pipette into disposable or well clean cuvettes :

	Blank	Standard	Sample
Reagent A	1000 µl	1000 µl	1000 µl
Distilled H ₂ O	10 µl		
Standard	-	10 µl	-
Sample	-	-	10 µl

Mix and incubate for **10 minutes** at **37 °C**.

Read the absorbance (A) of the standard and samples at **510 (500-546) nm** against Blank.

Colour is stable for 60 minutes, protected from light.

Note:

- Reaction volumes can be proportionally changed.
- For concentration > of 700 mg/dl (18.1 mmol/l) dilute sample 1:10 with NaCl (9 g/l) solution and multiply the result by 10.

CALCULATION OF RESULTS

$$\text{Cholesterol, mg/dl} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times 200$$

Conversion factor

$$\text{Cholesterol [mg/ml]} \times 0.02586 = \text{Cholesterol [mmol/l]}$$

REFERENCE VALUES

Cholesterol values according to a study on a population of adults in absence of coronary disease⁽³⁾ are the following:

Recommended values:	< 200 mg/dl (5.17 mmol/l)
Upper limit:	200÷239 mg/dl (5.2-6.2 mmol/l)
High values:	≥ 240 mg/dl (6.21 mmol/l)

Each laboratory should establish reference ranges for its own patients population.

ANALYTICAL PERFORMANCES

Precision

Within-run and between-run coefficients of variation have been calculated on replicates of three controls at different total cholesterol concentration. The obtained results are reported in the following table:

Sample	Mean (mg/dl)	Within-run		Between-run	
		SD	%CV	SD	%CV
Serum 1	32.5	0.57	1.8	1.02	3.1
Serum 2	93.7	1.35	1.4	3.08	3.3
Serum 3	205.9	4.41	2.1	5.45	2.6

Linearity

The assay is linear up to 700 mg/dl (18.1 mmol/l).

Sensitivity

Test sensitivity, in terms of limit of detection, is 4 mg/dl (0.103 mmol/l).

Correlation

A correlation study comparing the present method with a commercial one gave the following results:

$$y = 0.9971x + 3.9162 \text{ mg/dl} \quad r = 0.9898$$

Interferences

Bilirubin > 15 mg/dl
Hemoglobin > 500 mg/dl
Triglycerides > 1000 mg/dl

PRECAUTIONS IN USE**Standard is harmful (Irritant).**

Refer to Safety Data Sheet.

Reagent A is not considered harmful according to 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.

The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management

Please refer to local legal requirements.

BIBLIOGRAPHY

1. Jakobs DS, Kasten Jr. BL, Demmott WR, Wolfson WL: "Laboratory Test Handbook", Lexi-Comp and Williams & Wilkins Ed. 2nd Edition (1990).
2. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC: Enzymatic determination of total serum cholesterol. Clin Chem, 20:470 (1974).
3. Bachorik PS, Ross JW: National Cholesterol Education Program Recommendations for measurement of low-density lipoprotein cholesterol: executive summary. Clin Chem 41:1414-1420 (1995).
4. Chitto G, Fabi A, Franzini C, Galletta G, Leonardi A, Marelli M, Morelli AM: Variabilità biologica intra-individuo: rassegna della letteratura, contributo sperimentale e considerazioni critiche. Biochimica Clinica, 1994; 18, 10:673.
5. NCCLS Document, "Procedures for the collection of arterial blood specimens", Approved Standard, 3rd Ed. (1999).
6. EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC...
7. Kaplan LA, Pesce AJ: "Clinical Chemistry", Mosby Ed. (1996).

