COD 11805 1 x 50 mL	COD 11505 1 x 200 mL	COD 11506 1 x 500 mL	COD 11539 1 x 1 L
STORE AT 2-8ºC			
Reagents for measurement of cholesterol concentration Only for *in vitro* use in the clinical laboratory			

BioSystems

CE

CHOLESTEROL
CHOLESTEROL OXIDASE/PEROXIDASE

PRINCIPLE OF THE METHOD

Free and esterified cholesterol in the sample originates, by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry[1,2].

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{chol. esterase}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + \tfrac{1}{2}\,O_2 + H_2O \xrightarrow{\text{chol. oxidase}} \text{Cholestenone} + H_2O_2$$

$$2\,H_2O_2 + 4 - \text{Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4\,H_2O$$

CONTENTS

	COD 11805	COD 11505	COD 11506	COD 11539
A. Reagent	1 x 50 mL	1 x 200 mL	1 x 500 mL	1 x 1 L
S. Standard	1 x 5 mL	1 x 5 mL	1 x 5 mL	1 x 5 mL

COMPOSITION

A. Reagent. Pipes 35 mmol/L, sodium cholate 0.5 mmol/L, phenol 28 mmol/L, cholesterol esterase > 0.2 U/mL, cholesterol oxidase > 0.1 U/mL, peroxidase > 0.8 U/mL, 4-aminoantipyrine 0.5 mmol/L, pH 7.0.

S. Cholesterol Standard. Cholesterol 200 mg/dL (5,18 mmol/L). Aqueous primary standard.

STORAGE

Store at 2-8ºC.

Reagent and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

- Reagent: Presence of particulate material, turbidity, absorbance of the blank over 0.200 at 500 nm (1 cm cuvette).
- Standard: Presence of particulate material, turbidity.

REAGENT PREPARATION

Reagent and Standard are provided ready to use.

ADDITIONAL EQUIPMENT

- Thermostatic water bath at 37ºC
- Analyzer, spectrophotometer or photometer able to read at 500 ± 20 nm

SAMPLES

Serum or plasma collected by standard procedures.

Cholesterol is stable for 7 days at 2-8ºC. Heparin, EDTA, oxalate and fluoride may be used as anticoagulants.

PROCEDURE

1. Bring the Reagent to room temperature.
2. Pipette into labelled test tubes: (Note 1)

	Blank	Standard	Sample
Cholesterol Standard (S)	—	10 µL	—
Sample	—	—	10 µL
Reagent (A)	1.0 mL	1.0 mL	1.0 mL

3. Mix thoroughly and incubate the tubes for 10 minutes at room temperature (16-25ºC) or for 5 minutes at 37ºC.
4. Measure the absorbance (A) of the Standard and Sample at 500 nm against the Blank. The colour is stable for at least 2 hours.

CALCULATIONS

The cholesterol concentration in the sample is calculated using the following general formula:

$$\frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times C_{\text{Standard}} = C_{\text{Sample}}$$

If the Cholesterol Standard provided has been used to calibrate (Note 2):

$\frac{A_{\text{Sample}}}{A_{\text{Standard}}}$	x 200 = mg/dL cholesterol x 5.18 = mmol/L cholesterol

REFERENCE VALUES

The following uniform cut-off points have been established by the US National Cholesterol Education Program and have also been adopted in many other countries for the evaluation of coronary artery disease risk[3].

Up to 200 mg/dL = 5.2 mmol/L	Desirable
200-239 mg/dL = 5.2-6.21 mmol/L	Borderline High
> 240 mg/dL = > 6.24 mmol/L	High

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

- Detection limit: 0.3 mg/dL = 0.008 mmol/L
- Linearity limit: 1000 mg/dL = 26 mmol/L. For higher values dilute sample 1/2 with distilled water and repeat measurement.
- Repeatibility (within run):

Mean Concentration	CV	n
121 mg/dL = 3.13 mmol/L	1.1 %	20
257 mg/dL = 6.66 mmol/L	0.9 %	20

- Reproducibility (run to run):

Mean Concentration	CV	n
121 mg/dL = 3.13 mmol/L	1.9 %	25
257 mg/dL= 6.66 mmol/L	1.0 %	25

- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 2). Details of the comparison experiments are available on request.
- Interferences: Hemolysis (hemoglobin up to 500 mg/dL), bilirubin (up to 10 mg/dL) and lipemia (triglycerides up to 1000 mg/dL) do not interfere. Ascorbic acid (up to 6.25 mg/dL) does not interfere. Other drugs and substances may interfere[4].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

Cholesterol is a steroid of high molecular weight and possesses the cyclopentanophenanthrene skeleton. Dietary cholesterol is partially absorbed and it is also synthesized by the liver and other tissues. Cholesterol is transported in plasma by lipoproteins. It is excreted unchanged into bile or after transformation to bile acids.

Increased total cholesterol values are associated with a progressively escalating risk of atherosclerosis and coronary artery disease[5,6].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. This reagent may be used in several automatic analysers. Instructions for many of them are available on request.
2. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

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BioSystems S.A. Costa Brava, 30. 08030 Barcelona (Spain)
www.biosystems.global