CHOLESTEROL

Cat. No.	Pack Name	Packaging (Content)
BLT00034	CHOL 5x50	R1: 5 x 50 ml, R2 standard: 1 x 5 ml
BLT00035	CHOL 1000	R1: 1 x 1000 ml
BLT00036	CHOL 250	R1: 1 x 250 ml, R2 standard: 1 x 5 ml

EN

CE IVD

INTENDED USE
Diagnostic reagent for quantitative *in vitro* determination of Cholesterol in human serum and plasma.

CLINICAL SIGNIFICANCE
Measurement of serum cholesterol levels can serve as an indicator of liver function, biliary function, intestinal absorption, propensity towards coronary artery disease, thyroid function and adrenal disease. Cholesterol levels are important in the diagnosis and classification of hyperlipoproteinaemias. Stress, age, gender, hormonal balance and pregnancy affect normal cholesterol levels.

PRINCIPLE
This reagent is based on the formulation of Allain et al and the modification of Roeschlau with further improvements to render the reagent stable in solution.

1. Cholesterol ester + H_2O $\xrightarrow{CHE}$ Cholesterol + Fatty acids

2. Cholesterol + O_2 $\xrightarrow{CHO}$ Cholest-4-en-3-one + H_2O_2

3. $2H_2O_2$ + 4AAP + Phenol $\xrightarrow{POD}$ Quinoneimine dye + $4H_2O$

where:
CHE = Cholesterol Esterase
CHO = Cholesterol Oxidase
4AAP = 4-aminoantipyrine
POD = Peroxidase

1. Cholesterol esters are enzymatically hydrolysed by cholesterol esterase to cholesterol and free fatty acids.
2. Free cholesterol, including that originally present, then oxidized by cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide.
3. The hydrogen peroxide combines with 4-aminoantipyrine to form a chromophore (quinoneimine dye) which may be quantitated at 505 nm.

REAGENT COMPOSITION
R1

Good's Buffer	50 mmol/l
Phenol	5 mmol/l
4-aminoantipyrine	0.3 mmol/l
Cholesterol esterase	≥ 200 U/l
Cholesterol oxidase	≥ 50 U/l
Peroxidase	≥ 3 kU/l
R2 standard	See bottle label

REAGENT PREPARATION
Reagent is liquid, ready to use.

STABILITY AND STORAGE
The unopened reagents are stable till the expiry date stated on the bottle and kit label when stored at 2–8°C.

SPECIMEN COLLECTION AND HANDLING
Use serum, plasma (heparin, EDTA).
It is recommended to follow NCCLS procedures (or similar standardized conditions).
Stability

in serum / plasma:	at 20–25°C	7 days
	at 4–8 °C	7 days
	at -20°C	3 months

Discard contaminated specimens.

CALIBRATION
Calibration with the standard included in the kit or calibrator XL MULTICAL, Cat. No. XSYS0034 is recommended.

QUALITY CONTROL
For quality control ERBA NORM, Cat. No. BLT00080 and ERBA PATH, Cat. No. BLT00081 are recommended.

UNIT CONVERSION
mg/dl x 0.026 = mmol/l

EXPECTED VALUES [2]
Adult

Desirable blood Cholesterol	< 200 mg/dl
Borderline high blood Cholesterol	200 – 239 mg/dl
High blood Cholesterol	> 239 mg/dl

Child

Desirable blood Cholesterol	< 170 mg/dl
Borderline high blood Cholesterol	170 – 199 mg/dl
High blood Cholesterol	> 199 mg/dl

It is recommended that each laboratory verify this range or derives reference interval for the population it serves.

PERFORMANCE DATA
Data contained within this section is representative of performance on ERBA XL systems. Data obtained in your laboratory may differ from these values.

Limit of quantification: 4.2 mg/dl
Linearity: 695 mg/dl
Measuring range: 4.2 – 695 mg/dl

PRECISION

Intra-assay precision Within run (n=20)	Mean (mg/dl)	SD (mg/dl)	CV (%)
Sample 1	126.81	1.58	1.26
Sample 2	226.85	2.15	0.96

Inter-assay precision Run to run (n=20)	Mean (mg/dl)	SD (mg/dl)	CV (%)
Sample 1	106.46	1.12	1.06
Sample 2	187.46	3.08	1.65

COMPARISON
A comparison between XL-Systems Cholesterol (y) and a commercially available test (x) using 40 samples gave following results:

y = 0.995 x - 4.59 mg/dl
r = 1.000

INTERFERENCES
Following substances do not interfere:
haemoglobin up to 5 g/l, bilirubin up to 20 mg/dl, triglycerides up to 2000 mg/dl.

WARNING AND PRECAUTIONS
For *in vitro* diagnostic use. To be handled by entitled and professionally educated person.
Reagent of the kit is not classified like dangerous but contains less than 0.1% sodium azide - classified as very toxic and dangerous substance for the environment.

WASTE MANAGEMENT
Please refer to local legal requirements.

ASSAY PROCEDURE
Wavelength: 500 (546) nm
Cuvette: 1 cm

	Reagent blank	Standard (Calibr.)	Sample
Reagent 1	1.00 ml	1.00 ml	1.00 ml
Sample	-	-	0.01 ml
Standard (Calibr.)	-	0.01 ml	-
Distilled water	0.01 ml	-	-

Mix and incubate 10 min. at 37 °C. Measure absorbance of the sample A_{sam} and standard A_{st} against reagent blank. The coloration is stable during one hour.

CALCULATION
$$\text{Cholesterol (mg/dl)} = \frac{\Delta A_{sam}}{\Delta A_{st}} \times C_{st}$$

C_{st} = standard (calibrator) concentration

Applications for automatic analysers are available on request.

ASSAY PARAMETERS FOR PHOTOMETERS

Mode	End Point
Wavelength 1 (nm)	505
Wavelength 2 (nm)	670
Sample Volume (µl)	5/10
Reagent Volume (µl)	500/1000
Incubation time (min.)	5
Incubation temp. (°C)	37
Normal Low (mg/dl)	0
Normal High (mg/dl)	200
Linearity Low (mg/dl)	4.2
Linearity High (mg/dl)	695
Concentration of Standard	See bottle label
Blank with	Reagent
Absorbance limit (max.)	0.2
Units	mg/dl

Erba Lachema s.r.o., Karásek 1d, 621 00 Brno, CZ
e-mail: diagnostics@erbalachema.com, www.erbamannheim.com

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N/48/13/A/INT Date of revision: 27. 11. 2013

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SYMBOLS USED ON LABELS

REF Catalogue Number	Manufacturer	See Instruction for Use
LOT Lot Number	CE Mark - Device comply with the Directive 98/79/EC	Storage Temperature
Expiry Date	IVD In Vitro Diagnostics	CONT Content

QUALITY SYSTEM CERTIFIED
ISO 9001 ISO 13485

Erba Lachema s.r.o., Karásek 1d, 621 00 Brno, CZ
e-mail: diagnostics@erbalachema.com, www.erbamannheim.com