BIOLABO
www.biolabo.fr

MANUFACTURER:
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CHOLINESTERASE Butyrylthiocholine

Reagent for quantitative determination of Cholinesterase activity [EC 3.1.1.8] in human serum and plasma

REF 82526 **R1** 5 x 10 mL

CE

IVD

Made In France

TECHNICAL SUPPORT AND ORDERS
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Latest revision: www.biolabo.fr

I : correspond aux modifications significatives

I INTENDED USE

This reagent is designated for professional use in laboratory (manual or automated method).
It allows the quantitative determination of Cholinesterase activity in human serum or plasma to screen its level.

I GENERALITIES (1) (2)

Serum Cholinesterase (pseudocholinesterase, benzoil cholinesterase or cholinesterase II) is found in the liver, pancreas, heart, white matter of the brain and serum. Do not confused with the acetylcholinesterase (true cholinesterase or cholinesterase I) which is found in erythrocytes, lungs and spleen, nerve endings and grey matter of the brain.
The serum enzyme is the one whose assay is clinically useful, in the evaluation of liver function (impaired synthesis), atypical enzymes variants, and in the detection of possible insecticide poisoning.
Identification of patients with atypical form of the enzyme is important to prevent prolonged apnea caused by administration of succinylcholine anesthesia used in surgery.
Decreased levels of serum enzyme are also found in patients with acute infections, pulmonary embolism, and muscular dystrophy, as well as after surgical procedures.

PRINCIPLE (4) (5) (6)

Cholinesterase (SCH) catalyses the hydrolysis of butyrylthiocholine to thiocholine and butyric acid. Reaction scheme is as follows:

$$\text{Butyrylthiocholine} + H_2O \xrightleftharpoons{\text{SCH}} \text{Thiocholine} + \text{Butyric acid}$$

$$2\ \text{Thiocholine} + 2\ OH^- + 2\ \text{Hexacyanoferrate (III)} \longrightarrow \text{Dithiobis (choline)} + H_2O + 2\ \text{Hexacyanoferrate (II)}$$

The decrease in absorbance due to the conversion of Hexacyanoferrate (III) into Hexacyanoferrate (II), and proportional to SCH activity in the specimen, is measured at 405 nm.

REAGENTS

R1	**CHOLINESTERASE**	Working Reagent
Phosphate buffer pH 7.6	75	mmol/L
Hexacyanoferrate (III)	2	mmol/L
Butyrylthiocholine	15	mmol/L

ATTENTION (Before reconstitution):

Aquatic Chronic 3: Hazardous for the aquatic environment, long-term hazard, category 3, H412

Skin Irrit.2 : H315 - Causes skin irritation

STOT SE3 : H335 - May cause respiratory irritation

Eye Irrit.2 : H319 - Causes serious eye irritation

P280: Wear protective gloves/protective clothing/eye protection/face protection, P264 : Wash hands thoroughly after handling, 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 disposal regulations. Classification due to Butyrylthiocholine, iodure 25-<50%, Hexacyanoferrate(III) 2,5- < 10%. For more details, refer to Safety Data Sheet (MSDS)

Once reconstituted:

Working reagent is not classified as dangerous.

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

Reconstitute with 10 mL of demineralised water. Mix gently and wait for complete dissolution.

STABILITY AND STORAGE

Stored away from light, well caped in the original vial at 2-8°C, when stored and used as described, reagents are stable:

Unopened:

- Until expiry date stated on the label of the kit.

Once opened:

- Reconstitute immediately contents of vial R1.

Once reconstituted:

- Transfer requested quantity and store in the original vial at 2-8°C.
- Working reagent is stable for 2 weeks.
- Discard reagent if cloudy or if absorbance at 405 nm < 1.300.
- Don't use working reagent after expiry date.

SPECIMEN COLLECTION AND HANDLING (2)

Unhemolysed serum, heparinised or EDTA plasma.

SCH activity is stable in serum or plasma for:

- 7 days at 2-8°C.

LIMITS (3)

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

MATERIAL REQUIRED BUT NOT PROVIDED

1. Medical analysis laboratory equipment.
2. Spectrophotometer or Biochemistry Clinical Analyzer

QUALITY CONTROL

- REF 95516 HDL LDL CK-MB and lipids Control
- REF 95526 HDL LDL CK-MB and lipids Control
- 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 the tests 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)

Serum or plasma at 37°C	IU/L	µKat/L
Men	5900-12200	98-203
Women	4700-10400	78-173

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

PERFORMANCES

On Cobas Mira, 37°C, 405 nm

Linearity Range: between 123 and 25000 IU/L

Detection limit: approx. 123 IU/L

Precision:

Within-run N = 20	*Low level*	*Normal level*	*High level*
Mean (IU/L)	531	3674	5237
S.D. (IU/L)	30	37	45
C.V. %	5,6	1,0	0,9

Between run N = 20	*Low level*	*Normal level*	*High level*
Mean (IU/L)	654	4105	5454
S.D. IU/L	34	165	230
C.V. %	5,2	4,0	4,2

Comparison studies with commercially available reagent:
Realised on serum specimens (n=103) between 800 and 15000 IU/L

y = 0.9824 x + 151,4 r = 0.9962

Analytical Sensitivity: approx. 0.015 mAbs/min for 1 IU/L (1 cm pathlength, 405 nm)

Interferences:

Turbidity	No interference up to 0.383 abs
Total bilirubin	No interference up to 308 µmol/L

Due to cholinesterase released by Erythrocytes, haemolysis interferes with seric SCH Activity

Other substances may interfere (see § Limits)

On the board stability: 48 hours

Calibration Stability: 48 hours

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

CALIBRATION

- REF 95506: HDL LDL CKMB Calibrator traceable to *an Internal Masterlot*

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

PROCEDURE

Manual Procedure

Let stand reagents and specimens at room temperature.

Pipette into 1 cm path length thermostated cuvette (37°C):	
Reagent	1500 µL
Specimen	25 µL
Mix. After 90 seconds, record the absorbance at 405 nm every 30 sec during 90 sec.	
Calculate absorbance change per minute (ΔAbs/min).	

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

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

CALCULATION

With Serum Multicalibrator

$$\text{SCH Activity} = \frac{(\Delta\text{Abs/min) Assay}}{(\Delta\text{Abs/min) Calibrator}} \times \text{Calibrator Concentration}$$

With theoretical factor:

Activity (IU/L) = ΔAbs/min x Factor

$$\text{Factor} = \frac{\text{VR} \times 1000}{0.9118 \times \text{VE} \times \text{P}}$$

With:
VR = Total reactional volume (mL)
VE = Specimen volume (mL)
0,912 = Molar extinction coefficient for Hexacyanoferrate III at 405 nm
P = Path length (cm).

Example, with manual Procedure,
(Path length 1 cm, 37°C, 405 nm):

IU/L = (ΔAbs/min) x 65804

$$\text{µkat/L} = \frac{\text{IU/L}}{60}$$

REFERENCES

(1) *TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 708-711*
(2) *Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p. 250-251.*
(3) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p. 3-68 to 3-79*
(4) *DGKC. Proposal of standard methods for the determination of enzyme catalytic concentrations in serum and plasmas at 37°C. II Cholinesterase. Eur J Clin Chem Chim Biochem 1992; 30 / p.163-170.*
(5) *Pantheghini . and Bonora R. Evaluation of a new continuous colorimetric method for determination of serum pseudo-cholinesterase activity and its application to a centrifugal fast analyzer. J Clin Chem Clin Biochem 1984; 22: p.671-676.*
(6) *Whittaker M., Britten J.J., and Dawson P. J. Comparison of a commercially available system with two reference methods for the etermination of plasma cholinesterase variant. Clin Chem 1983; 29: p.1746-1751*

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