



CHOLINESTERASE LR liquid reagent

REF C2500420 364 Test

CE C2500420A 364 Test
IVD For in vitro medical device

Use

Kit for the quantitative in vitro diagnostic determination of cholinesterase in serum or plasma. Colorimetric optimized method DGKC.

Summary

Cholinesterase activity measurements are useful as a test of liver function. And in the monitoring of surgical patient with an impairment in their ability to metabolise succinylcholine.

Principle

Cholinesterase catalyzes the butyrylthiocholine hydrolysis at thiocholine in presence of potassium hexacyanoferrate (III). The reduction absorbance velocity is proportional to the cholinesterase activity in the sample.

Reagents

R1	Pirophosphate	pH 7.3	90.0 mmol/l
	hexacyanoferrate (III)		3.0 mmol/l
R2	Goods buffer	pH 5.5	20.0 mmol/l
	butyrylthiocholine iodide		72.0 mmol/l

Reagent Preparation

Reagents are liquid and ready to use. About using as monoreagent ("sample-starter") dissolve the content of R2 vial in the R1 vial. For minor use add to every 4 ml of R1 reagent, 1 ml of R2 reagent. Keep out the reagents from refrigerator only for the use and recap them immediately.

Storage And Stability

- Store the kit at 2-8°C. Do not freeze the reagents.
- After opening, the vials R1 and R2 are stable 90 days if recapped immediately and protected from contamination, evaporation, direct light, and stored at the correct temperature.
- Working solution stability (R1+ R2): 14 days at 2-8°C.

Precaution In Use

The product is not classified as dangerous (DLg. N. 285 art. 28 l. n. 128/1998). However the reagent should be handled with caution, according to good laboratory practice.

Waste Management

Please refer to the local legal requirements.

Sample

- Serum -heparinized plasma or EDTA plasma.
- Do not use samples with haemolysis.
- Separate, as soon as possible, serum and plasma from red cells after picking because the cholinesterase activity can increase till 25% if the serum is left one day into contact with the corpuscolate part.
- Do not use sodium fluoride as anticoagulant because inhibit the enzyme activity.
- The cholinesterase is stable in the samples up to 15 days at 2-8°C

Note

- The kit, according to this method, must be used in manual procedures. About automatic using follow specific applications.
- Avoid direct light, contamination and evaporation.
- The volumes in the procedure can be changed proportionally.
- In case of complaint or quality control request, refer to the lot number on the package or the lot number on the single vials.

Procedure

Wavelength	λ: 405 (400 – 440) nm
Working Temperature	37°C
Optical Path	1 cm
Reaction	"kinetic"

Bring the reagent at 15-25°C before using it

Monoreagent Procedure "sample starter"

	Blank	Sample
Working Reagent	1000µl	1000µl
Distilled Water	15 µl	-
Sample	-	15 µl

Mix, then incubate 1' at 37°C. Measure the absorbance of sample (EC) and blank (EB) in decreasing after 30", 60", 90". Calculate the ΔE/min.

Bireagent Procedure "substrate starter"

	Blank	Sample
Reagent R1	800 µl	800 µl
Distilled Water	15 µl	-
Sample	-	15 µl

Mix, incubate at 37°C for 1' and then add:

	Blank	Sample
Reagent R2	200 µl	200 µl

Mix, then incubate 1' at 37°C. Measure the absorbance of sample (EC) and blank (EB) in decreasing after 30", 60", 90". Calculate the ΔE/min.

Calculation

$$\text{Cholinesterase [U/l]} = (\Delta E/\text{min C} - \Delta E/\text{min B}) \times 62000$$

The reagents performances are related to 37°C, 1 cm and 405 nm.

Reference Values

Adults	3000 – 9000 U/l
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Reference values are considered indicative since each laboratory should establish reference ranges for its own patient population. The analytical results should be evaluated with other information coming from patient's clinical history.

Analytical Performances

Linearity

Reaction is linear up to a concentration of 12000 U/l. Samples with values exceeding 12000 U/l must be diluted with saline solution. Then, multiply, the result for diluting factor.

"Intra-Assay" precision (within-Run)

Determined on 20 samples for each control (N-H) (Normal-High). Results:

MEAN (U/l)	N = 4965.50	H = 2723.00
S.D.	N = 69.7	H = 26.48
C.V.%	N = 1.40	H = 0.97

"Inter-Assay" precision (between-Run)

Determined on 20 samples for each control (N-H). Results:

MEAN (U/l)	N = 4894.67	H = 2716.83
S.D.	N = 51.56	H = 29.97
C.V.%	N = 1.05	H = 1.10

Analytical sensitivity

The test sensitivity in terms of detection limit is 85 U/l.

Correlation

A study based comparing this method with a similar method on 2 samples has given a correlating factor $r = 0.99$

$$y = 0.9823x + 365.85$$

Interferences

No interference was observed by the presence of Triglycerides ≤ 800 mg/dl
Bilirubin ≤ 25 mg/dl
Do not use haemolysed sample.

Quality Controls

It's necessary, every time the kit is used, to make the quality controls and to check that values obtained are within the acceptance range provided in the insert. Each laboratory should establish its own mean and standard deviation and adopt a quality control program to monitor laboratory testing.

Bibliography

Kaplan, L.A., Pesce, A.J.: "Clinical Chemistry", Mosby Ed. (1996).
Knedel B., Boettger R., Klin. Wschr., 45, 325, (1967).
Deutsche Gesellschaft für Klinische Chemie: "Proposal of Standard Methods for the determination of enzyme catalytic concentrations in serum and plasma at 37°C, II. Cholinesterase (Acylocholine Acylhydrolase)". Eur. J. Clin. Chem. Clin. Biochem. 30, 163 (1992).

Symbols

CE CE Mark (requirement of 98/79 regulation)

IVD in vitro medical device

LOT Batch Code

Use by

Storage temperature limits

Read instruction for use

Gesan Production srl

Cholinesterase LR
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Gesan Production s.r.l

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