

# CHOLINESTERASE LIQUID



KINETIC COLORIMETRIC DETERMINATION IN SERUM AND PLASMA  
 BUTYRILTHIOCHOLINE (BTC) METHOD (DGKC RECOMMENDATION)  
 For in vitro diagnostic use only

Kit: 4 x 60 ml

Cod. PCB261

## SUMMARY

Cholinesterase levels in serum are useful for the evaluation of liver function, as an indicator of (insecticide) poisoning or for the detection of patients with atypical forms of the enzyme. Decreases are observed in acute hepatitis, cirrhosis and carcinomas; in acute infections, muscular dystrophy. Severe inhibition of the functionality occurs with organic phosphorus compounds (organic insecticide) able to give a deep poisoning. A few drugs used in surgery as muscle relaxant are metabolized as the Cholinesterase one; with low level of Cholinesterase or with atypical enzyme variants, patients may enter in a period of prolonged apnea. Preoperative screening is suggested to identify patients who may lead to complications.

## PRINCIPLE

The cholinesterase present in the serum catalyzes the hydrolysis of the butyrylthiocholine (BTC), forming butyrate and thiocholine. The thiocholine reduces the exacyanoferrate (III) to exacyanoferrate (II). The **decrease** of absorbance in the unit time at 405 nm is proportional at the activity of the cholinesterase in the sample.

## REAGENTS

Components of the kit:

Cod.PCB261

\*REAGENT 1 (liquid) 4 x 50 mL  
 \*REAGENT 2 (liquid) 4 x 10 mL  
 Buffer pyrophosphate 75 mmol/L  
 Exacyanoferrate (III) > 0.5 mmol/L  
 BTC 15 mmol/L

STABILITY: the reagents, stored at 2-8°C, are stable up to the expiry date shown on the package if **not contaminated during handling**.

## AUXILIARY REAGENTS FOR CALIBRATION and for QUALITY CONTROL (Not supplied with the kit)

We suggest strongly to calibrate always on the instruments.

To grant a good calibration we suggest to use following kit:

- CALIBRATOR Cod. CALFAS3

To grant the correct test performances we suggest to use following kits:

- NORMAL CONTROL Cod. CNU  
 - PATHOLOGICAL CONTROL Cod. CPU

## PREPARATION OF THE WORKING REAGENT

Ready to use.

Mix kindly and let the working reagent reaches the working temperature before use. **Close immediately after handling.**  
**Incompetent handling will release us from any responsibility.**

## SAMPLE

• No haemolyzed serum, plasma with heparin or EDTA.

## PROCEDURE

• Wavelength: 405 nm  
 • Pathlength: 1 cm  
 • Reading: against air or distilled water  
 • Temperature: 37°C  
 • Method: kinetic  
 • Reaction: 90 seconds  
 • Linearity: up to 25 kU/l a 37°C  
 • Sample/Reagent: 1/50/10

Let reagent reaches the working temperature before using.

Pipette into a test tube or cuvette labelled:

	R/B	S
*Reagent 1	1000 µl	1000 µl
Distilled water	20 µl	---
Sample	---	20 µl

Mix and incubate for about 5 minutes at 37°C. Then add:

*Reagent 2	200 µl	200 µl
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Mix carefully and measure the absorbance against air or distilled water. Repeat the reading after 30 seconds for 3 times. Determine the average of the readings ( $\Delta A/30$  sec).

## CALCULATION

Replace the average ( $\Delta A/30$ sec. Sample -  $\Delta A/30$ sec. Reagent Blank) found in the following formula:

Cholinesterase (KU/L) =  
 = ( $\Delta A/30$ sec. Sample -  $\Delta A/30$ sec. Reagent Blank) x 131.6

## REFERENCE VALUES

4,6 - 13 KU/L at 37°C

It is advisable that every laboratory determines its normal reference values.

## PERFORMANCE CHARACTERISTICS

These performance characteristics was determined using a spectrophotometer or analyzers typically found in clinical laboratories, under the stated assay conditions.

**Linearity:** The Cholinesterase may be determined between 250–25000 U/L.

Dilute the sample with activity higher than 25000 U/L, with saline solution 1:4 repeat the determination and multiply the result by 4.

**Sensitivity:** The minimum detectable is 250 U/L.

## Within-run Precision:

	Mean (U/L) ± 2s	CV %
Serum 1	1775,30 ± 71,5	2,01
Serum 2	5369,0 ± 132,9	1,24

## Run-to-run (Day-to-day) Precision:

	Mean (U/L) ± 2s	CV %
Serum 1	1753,13 ± 126,3	3,60
Serum 2	5314,3 ± 186,5	1,75

## Accuracy: with commercially available Control(s)

	Waited (U/L)	Found (U/L)
normal	7630 (6256-9004)	7019-7324-7950
pathological	6610 (5419 - 7801)	6081-6345-6887

**Interferences:** See References point 2.

**Correlation:** A group of 20 sera was assayed by this procedure and using a similar commercially available Cholinesterase Reagent. Comparison of the data gave following results:

Linear regression equation  $y = 1,0041x - 31$   
 Correlation coefficient  $r = 0,9978$



## NOTE

1. A proportional variation of the reaction volumes does not change the result.
2. We suggest do not mix Reagents from different Production lots.
3. Dilute the sample with activity higher than 25000 U/L ( $\Delta A/30\text{sec.} > 0.190$ ), with saline solution 1:4 repeat the determination and multiply the result by 4.
4. Avoid the use of anticoagulants containing fluorides.
5. Very deep attention must be given to interfering substances: certain drugs and other substances are able to influence levels of Cholinesterase (see References 2.).
6. PAY ATTENTION!  
Applications on routine Analyzers may be totally different from what we developed as manual determination, and also from themselves.
7. The reagent must be used only for the intended destinations, by expert people and in the due lab. conditions.
8. The clinical diagnosis cannot be done using the result of only one test, but have to be done integrating different lab. and clinical data.

## REFERENCES

1. Textbook of Clinical Chemistry, Ed. by N.W. Tietz, W.B. Saunders Co., Philadelphia (1999).
2. Young D.S. et al., Clin. Chem. 21, 302D (1975).
3. DGKC (German Society for Clinical Chemistry) Eur. J. Clin. Chem. Clin. Biochem. 30/3, 163 (1992).

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