



CHOLINESTERASE - L

DGKC kinetic colorimetric method for the determination of Cholinesterase activity in serum and plasma



ORDER INFORMATION

REF	Kit size
GA4975 00	2x40 + 1x20 ml
KL4975 00	1x48 + 1x12 ml
BK4975 00	2x(50+13 ml)

INDICATION

The term Cholinesterase indicates a group of enzymes having in common the capacity of hydrolyze choline esters. Cholinesterase is synthesized by the liver, for this reason the determination of its activity is indicated for diagnosis and control of hepatic diseases. Cholinesterase activity results slightly decreased in chronic and acute hepatitis; the decrease results more accentuate in case of hepatic cirrhosis. Decrease in enzyme activity is also observed in case of hepatic cells carcinoma, biliary ducts diseases, grave states of proteic deficiency, cachexia, chronic infections, acute paramyeloblastic leukemia and in case of phosphoric ester poisonind (e.g. pesticides).

Elevated values of cholinesterase activity are revealed in case of hepatic steatosis caused by alcoholism without inflammatory activity, and in case of diseases with increased synthesis of albumin (e.g. nephrosis, exudative enterophaty and thyrotoxicosis).

Cholinesterase activity determination must be executed before treating patients with the muscle relaxant succinylthiocholine. If enzyme activity is decreased, both for hepatic damages and genetic causes, this substance can cause prolonged apnea.

METHOD PRINCIPLE

In the present method (DGKC), Cholinesterase catalyzes the hydrolysis of butyrylthiocholine into butyrate and thiocholine. Thiocholine reduces ferricyanide ion into ferrocyanide. Absorbance value at 405 nm decreases proportionally to the enzyme activity in the sample.

COMPOSITION

REAGENT A:

Sodium pyrophosphate	75 mmol/l
Potassium esacyanoferrate III	2 mmol/l
Sodium azide	0.095%

REAGENT B:

Good buffer, pH 4.5	25.3 mmol/l
Butyrylthiocholine	400 mmol/l

PREPARATION OF REAGENTS

Bireagent procedure:

The reagents are liquids ready to use.

Monoreagent procedure:

Mix 4 parts of Reagent A and 1 part of Reagent B to obtain the working reagent (ex. 20 ml of RA + 5 ml of RB).

Storage and stability

Store at 2-8 °C. Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature

Working reagent is stable for 3 days if stored at 2-8 °C.

ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l

SAMPLES

Serum, plasma not hemolyzed serum. Do not utilize sodium fluoride as anticoagulant as it inhibits enzyme activity. Immediately separate serum or plasma from erythrocytes as they contain cholinesterase. Cholinesterase activity increases of about 25-30% a day if serum or plasma are in contact with red blood cells.

Stable 1 month at 2-8°C.

Specimen collection / Preanalytical factors

It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

INTERNAL QUALITY CONTROL

It is recommended to use commercial Quality Control sera with known Cholinesterase activities. Check that the values obtained are within the reference range provided.

ANALYTICAL PROCEDURE

Working temperature	37 °C
Wavelength	405 nm (400-410 nm)
Optical path	1 cm
Reaction	kinetic (decrease)

Allow the reagents to reach working temperature before using.

Bireagent procedure

Pipette into disposable or well clean cuvettes:

	Blank	Sample
Reagent A	-	800 µl
Distilled H ₂ O	1000 µl	-
Sample	-	15 µl
Mix and incubate 5 minutes at 37 °C, then add:		
Reagent B	-	200 µl
Mix and incubate 1 minute at 37 °C. Read initial absorbance and repeat absorbance reading after 1, 2, 3 minutes against blank. Calculate ΔA/minute.		

Monoreagent procedure

Pipette into disposable or well clean cuvettes:

	Blank	Sample
Working reagent	-	1000 µl
Distilled H ₂ O	1000 µl	-
Sample	-	15 µl
Mix and incubate 1 minute at 37 °C. Read initial absorbance and repeat absorbance reading after 1, 2, 3 minutes against blank. Calculate ΔA/minute.		

CALCULATION OF RESULTS

Cholinesterase U/l = ΔA/min. x 62000

REFERENCE VALUES

Male 5600 ÷ 11200 U/l
 Female 4200 ÷ 10800 U/l

Each laboratory should establish reference ranges for its own patients population.

ANALYTICAL PERFORMANCES

Precision

Within-run and between-run coefficients of variation have been calculated on replicates of three samples at different enzymatic activity. The obtained results are reported in the following table:

Sample	Mean (U/l)	Within-run		Between-run	
		SD	%CV	SD	%CV
Serum 1	3989	110.3	2.76	288.5	7.23
Serum 2	2245	40.8	1.82	133.5	5.95
Serum 3	1848	27.1	1.47	95.6	5.17

Linearity

The assay is linear up to 12000 U/l.

Sensitivity

Test sensitivity, in terms of detection limit, is 120 U/l.

Correlation

A study based comparing this method with a commercial one gave the following results:

$$y = 1.0448x - 79.36 \text{ U/l} \quad r = 0.9388$$

Interferences

Hemoglobin > 200 mg/dl
 Bilirubin > 20 mg/dl
 Triglycerides > 1000 mg/dl

PRECAUTIONS IN USE

The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/ECC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.

The use of the laboratory reagents according to good laboratory practice is recommended.

Waste Management

Please refer to local legal requirements.

BIBLIOGRAPHY

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