



DIBUCAINE REAGENT

Auxiliary reagent for the determination of Dibucaine number in serum and plasma



ORDER INFORMATION

REF	Kit size
GD0971 00	5x20 ml
KL0971 00	1x60 ml
BK0971 00	2x40 ml

INDICATION

Cholinesterase in the serum can be present in the normal form and in another form called atypical. Only normal cholinesterase is strongly inhibited by dibucaine. The determination of inhibition grade (Dibucaine Number, DN) allows to relieve the presence of atypical form.

The determination of cholinesterase activity inhibition must be executed before treating patients with the muscle relaxant succinylthiocholine to identify carrier subjects of the atypical variant. In these subjects administration of succinylthiocholine 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 D (powder):
Dibucaine hydrochloride 0.1 mmol/l
Excipients and preservatives

PREPARATION OF REAGENTS

Working reagent preparation:

Utilize Reagent A and Reagent B of the Cholinesterase-L Ref. GA4975 00 kit. The reagents are liquids ready to use.

Mix 4 parts of Reagent A and 1 part of Reagent B.

Working reagent with inhibitor preparation:

Dissolve the content of Reagent D bottle with 20 ml of working reagent.

Storage and stability

- Reagent A, Reagent B and Reagent D are stable up to expiry date stated on the labels if stored at 2-8 °C.
- Working reagent (RA+RB) is stable 3 days if stored at 2-8 °C.
- Working reagent with inhibitor (RA+RB+RD) is stable 3 days if stored at 2-8 °C.

Do not freeze the reagents! 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, protected from light.

ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l
- Cholinesterase-L Ref. GA4975 00

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.

Total Cholinesterase activity determination

Pipette into disposable or well clean cuvettes:

	Blank	Sample
Working reagent (RA+RB)	-	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.

Cholinesterase activity with inhibitor determination

Pipette into disposable or well clean cuvettes:

	Blank	Sample
Working reagent with inhibitor (RA+RB+RD)	-	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

Determine total Cholinesterase activity and Cholinesterase activity with inhibitor according to the following formula:

$$\text{Cholinesterase U/l} = \Delta A/\text{min.} \times 62000$$

Calculate Dibucaine Number (DN) as follows:

$$\text{DN} = 100 - \frac{\text{Activity with inhibitor (U/l)}}{\text{Total activity (U/l)}} \times 100$$

REFERENCE VALUES

Normal: DN > 75%
Heterozygotes: DN 35÷75%
Homozygotes: DN < 35%

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

ANALYTICAL PERFORMANCES

Refer to the data reported in Cholinesterase-L Ref. GA4975 00 kit package insert kit.

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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4. Tiets NW,: Clinical guide to laboratory tests, 2nd ed., Saunders Co., (1991).
5. NCCLS Document, "Procedures for the collection of arterial blood specimens", Appr. Std., 3rd Ed. (1999).
6. EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC.