



# DIBUCAINE (Dibucaine Number)

## Colorimetric determination of Dibucaine Number

### TEST SUMMARY

The Cholinesterase in serum can be present even in normal form and even as atypical variant. Only the normal Cholinesterase is strongly inhibited by dibucaine.

The determination of inhibitor level (Dibucaine Number) allows to underline the presence of the atypical variant of Cholinesterase.

### SAMPLES

Serum, plasma (EDTA or heparine). Do not utilize sodium fluorure as anticoagulant as it inhibits enzyme activity. Avoid the haemolysis. Immediately separate serum or plasma from erythrocytes as they contain cholinesterase. Cholinesterase activity increase of about 25-30% a day if serum or plasma are in contact with red blood cells.

Stability: 1 month at 2-8°C, 7 days at 15-25°C.

### REAGENTS

Dibucaine Reagent: powder, dibucaine chloroHydrate 0.1 mmol/l; excipient, preservatives.

### MATERIAL REQUIRED BUT NOT SUPPLIED

AUXILIARY REAGENT REF. CC01200.

Normal laboratory equipment. Spectrophotometer UV/VIS with thermostataion. Automatic Micropipette. Cuvette in optical glass or monouse in optical polystyrene. Distilled water.

### PRECAUTIONS

Reagent may contain not reactive and conservative components. It is opportune to avoid contacts with the skin and do not swallow.

Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

### REAGENTS PREPARATION

AUXILIARY REAGENT.

Add 10 ml of Reagent B to a vial of Reagent A. Stability of Work Reagent: 3 weeks at 2-8°C away from light source.

Reagent A and Reagent B are stable until expiration date on label away from light source at 2-8°C. Stability after first opening: ≥ 60 days at 2-8°C.

Dissolve one vial of Dibucaine Reagent with 20 ml of Work Reagent.

Stability: 3 weeks at 2-8°C away from light source.

### PROCEDURE

Kind of analysis: Kinetics (decrease)  
Reading time: 60, 120, 180, 240 sec.  
Wavelength: 405 nm  
Temperature: 37°C  
Lightpath: 1 cm  
Zero: Distilled water

REAGENTS	CUVETTE
Work reagent	1000 µl
Preincubate at 37°C for 5 minutes	
Sample	15 µl
Mix, after 60 seconds measure the absorbance against water, incubate at 37°C. Execute other 3 readings at 60 seconds distance. Calculate the $\Delta A/\text{min}$ .	

### CALCULATION

#### Serum/Plasma

- Determine the **total activity** of Cholinesterase with auxiliary kit.
- Determine the **activity of Cholinesterase with inhibitor**.

#### Sericeous cholinesterase total (U/l)

$$\Delta A/\text{min} \times 62000$$

#### Calculate the Dibucaine Number DN

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

### EXPECTED VALUES

Total Cholinesterase

Men 5600 – 11200 U/l  
Women 4200 – 10800 U/l

Dibucaine number:  
Normal homozygous > 75 %  
heterozygous 35 – 75 %  
Atypical homozygous < 35 %

Every laboratory should establish own reference intervals in accordance with own population.

### NOTES

- Use only with Cholinesterase Kit (cod. CC01200)**
- If the results are incompatible with clinical presentation, they have to be evaluated within a total clinical study.
- Only for IVD use.

### CALIBRATIN/QUALITY CONTROL

It is suggested to perform an internal quality control. For this purpose the following control sera on human base are available on request:

**QN 0050 CH** 10 x 5 ml  
Control Sera normal values

**QP 0050 CH** 10 x 5 ml  
Control Sera pathological values

### TEST PERFORMANCE

#### Precision

Reproducibility studies of intra and inter assay precision gave  $CV\% < 1.5$ .  
A correlation study with similar commercial method gave the following results:  $y = 1,0107x - 79,75$ ;  $r = 0.9995$ .

#### Sensibility/limit of detenction

The method is able to discriminate up to 432.3 U/l.

#### Linearità

The method is linear up to 15000 U/l.  
If  $\Delta A/\text{min}$  is exceeded to 0.25 is suggested to dilute the sample 1+9 with physiological solution and perform again the test, multiplying the results by 10.

#### Interferences

No interference was observed by the presence of:

hemoglobin ≤ 180 mg/dl  
Triglycerides ≤ 1000 mg/dl

### WASTE DISPOSAL

Product is intended for professional laboratories. Waste products must be handled as per relevant security cards and local regulations.

### PACKAGING

#### CODE CC01270

Dibucaine Reagent 5 x 20 ml (powder)

### REFERENCES

Kalow W., Genesi K., Can. J. Biochem. Physiol. 35:339-346 (1975).  
Garry P., Clin. Chem. 17(3):183-191 (1971).  
EU-Dir 1999/11 Commission Directive of March 1999 adapting to technical progress the principle of good laboratory practice as specified in Council Directive 87/18/EEC.

### MANUFACTURER

LTA s.r.l.  
Via Milano 15/F  
20060 Bussero (Milan) ITALY  
Tel: ++39 02 95409034  
Fax: ++39 02 95334185  
e-mail: info@ltaonline.it  
Website: http://www.ltaonline.it

### SYMBOLS

- Only for IVD use
- Lot of manufacturing
- Code number
- Storage temperature interval
- Expiration date (year, month)
- Warning, read enclosed documents
- Read the directions
- Biological risk

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