DIBUCAINE REAGENT

CH F080 CH to prepare 4 x 20 ml

INTENDED USE

Reagent for determination of dibucaine number, to be used in quantitative in vitro assay of cholinesterase in biological fluids.

SUMMARY OF TEST

Measurements of total serum cholinesterase activity as well as determination of the "dibucaine number" are needed to fully characterize cholinesterase variants. The latter parameter indicates the percentage inhibition of enzyme activity toward specified substrates in the presence of a standard concentration of inhibitor.

PRINCIPLE OF THE METHOD

This reagent is formulated according to DGKC reccomendations. Cholinesterase (pseudocholinesterase EC 3.1.1.8) catalyzes the hydrolysis of butyrylthiocholine, forming butyrate and thiocholine, which reduces the ferricyanide ions to ferrocianyde.
The decrease in absorbance is followed at 405 nm and it is proportional to cholinesterase activity in examined sample. Cholinesterase activity is measured with and without presence of dibucaine as inhibitor. Dibucaine number is calculated on the basis od inhibition percentage.

KIT COMPONENTS

For in vitro diagnostic use only.
The components of the kit are stable until expiration date on the label.
Keep away from direct light sources.

DIB R1: 4 x 0.2 ml (liquid)
Composition: dibucaine hydrochloride 50 mM.

Store all components at 2-8°C.

MATERIALS REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micropipettes. Glass or high quality polystyrene cuvettes. Saline solution.
CHOLINESTERASE FL reagent (codes CH F096 CH or CH F245 CH).

REAGENT PREPARATION

Cholinesterase (total):
use separate reagents ready to use (kit CHOLINESTERASE FL, not supplied).
Stability: up to expiration date on labels at 2-8°C;
Stability since first opening of vials: preferably within 60 days at 2-8°C.
Cholinesterase (inhibited):
add 0,2 ml of DIB R1 to 20 ml of CHE R1 of kit CHOLINESTERASE FL (not supplied).
Stability: 30 days at 2-8°C;

PRECAUTIONS

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.
Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

SPECIMEN

Serum, plasma (EDTA, heparinate only). Avoid hemolysis. ChE is stable in sample for at least 14 days whether the sample is stored at room temperature or under refrigeration.

TEST PROCEDURE (cholinesterase total)

Wavelenght:	405 nm
Ligthpath:	1 cm
Temperature:	37°C
dispense in cuvette CHE R1 without dibucaine:	1 ml
add sample:	20 µl
incubate at 37°C for 5 minutes.	
dispense in cuvette CHE R2:	200 µl

Mix, execute a first reading of absorbance after 90 seconds, incubating at 37°C. Perform other 3 readings at 30 seconds intervals. Calculate the ΔA/min.

TEST PROCEDURE (cholinesterase inhibited)

Wavelenght:	405 nm
Ligthpath:	1 cm
Temperature:	37°C
dispense in cuvette CHE R1 with dibucaine:	1 ml
add sample:	20 µl
incubate at 37°C for 5 minutes.	
dispense in cuvette CHE R2:	200 µl

Mix, execute a first reading of absorbance after 90 seconds, incubating at 37°C. Perform other 3 readings at 30 seconds intervals. Calculate the ΔA/min.

RESULTS CALCULATION

Perform calculation in units per litre, multiplying the ΔA/min by the factor as it is indicated.

CHE total activity U/l: ΔA/min x 65800
CHE inhibited activity U/l: ΔA/min x 65800

Dibucaine number calculation (DN):

DN = 100 - [(U/l CHE inhibited / U/l CHE total) x 100]

EXPECTED VALUES

Total SChE:
Men: 5600 - 11200 U/l
Women: 4200 - 10800 U/l

Dibucaine number:
Normal homozygotes: > 75%
Heterozygotes: 35 - 75%
Atypical homozygotes: < 35%

Each laboratory should establish appropriate reference intervals related to its population.

QUALITY CONTROL AND CALIBRATION

It is suggested to perform an internal quality control. For this purpose the following human based control sera are available:
QUANTINORM CHEMA
with normal or close to normal control values
QUANTIPATH CHEMA
with pathological control values.
If required, a multiparametric, human based calibrator is available:
AUTOCAL H

Please contact Customer Care for further information.

TEST PERFORMANCE

Linearity
the method is linear up to 25000 U/l.
If a ΔA/min of 0.30 is exceeded, it is suggested to dilute sample 1+9 with saline solution and to repeat the test, multiplying the result by 10.

Sensitivity/limit of detection (LOD)
the limit of detection is 432.3 U/l.

Interferences
no interference was observed by the presence of:

hemoglobin	≤ 500 mg/dl
bilirubin	≤ 40 mg/dl
lipids	≤ 800 mg/dl

Precision

intra-assay (n=10)	mean (U/l)	SD (U/l)	CV%
sample 1	5972.9	122.8	2.1
sample 2	5743.8	57.5	1.0

inter-assay (n=20)	mean (U/l)	SD (U/l)	CV%
sample 1	5808.4	113.4	2.0
sample 2	5753.5	99.6	1.7

Methods comparison
a comparison between Chema Cholinesterase FL and a commercially available product gave the following results:

SChE Chema = x
SChE competitor = y
n = 107

y = 0.985x + 51.7 U/l r^2=0.996

WASTE DISPOSAL

This product is made to be used in professional laboratories.
P501: Dispose of contents according to national/international regulations.

REFERENCES

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Anestesiology, 91:1798-1806 (1999)
Clin. Chem., 19:1309-1313 (1973)
Tietz Textbook of Clinical Chemistry, Second Edition, Burtis-Ashwood (1994).

MANUFACTURER

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SYMBOLS

IVD	*in vitro* diagnostic medical device
LOT	batch code
REF	catalogue number
	temperature limit
	use by date
	caution
	consult instructions for use

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