Cholinesterase Reagent

Cholinesterase Reagent

Order No.	Description
R85136	20 x 6.5 mL

INTENDED USE

This reagent is intended for the in vitro measurement of the activity of cholinesterase in serum.

CLINICAL SIGNIFICANCE

Acetylcholinesterase hydrolyzes acetylcholine released at nerve endings to mediate transmission of neural impulses across the synapse.

The assay of the enzyme cholinesterase in serum is clinically useful. This enzyme acts on a variety of choline and thiocholine esters. It is synthesized in the liver and the assay of its activity in serum is a sensitive test of liver function (1, 2, 3).

Acetylcholinesterase and serum cholinesterase are irreversibly inhibited by some organic phosphorus compounds including the insecticides malathion and parathion. The assay for serum cholinesterase is a sensitive test of exposure of farm workers to these insecticides.

The muscle relaxant succynilcholine acts by inhibiting acetylcholinesterase but is rapidly hydrolyzed by serum cholinesterase. Thus its action is of short duration; for this reason, the drug is widely employed in surgical procedures. However, some patients with genetic variants of serum cholinesterase have lower than normal enzyme activity levels, therefore, the drug is hydrolyzed very slowly. This results in prolonged apnea lasting hours following administration of the drug. Determination of the enzyme in serum and the degree of activity inhibition by dibucaine and fluoride is important in discovering individuals with enzyme variants before surgery (4, 5, 6).

TEST SUMMARY

Colorimetric methods for serum cholinesterase assays employ esters of thiocholine. In our assay the substrate is butyrylthiocholine. The enzyme hydrolyzes this substrate to butyrate and thiocholine. Thiocholine reacts with 5,5'-dithiobis-2-nitrobenzoic acid (Ellman's reagent: DTNB) (7) to form 5-mercapto-2-nitrobenzoic acid (5-MNBA) which has an intense yellow color.

The reaction is as follows:

$$\text{Butyrylthiocholine} + H_2O \xrightarrow{\text{Cholinesterase}} \text{Butyrate} + \text{Thiocholine}$$

$$\text{Thiocholine} + \text{DTNB} \longrightarrow \text{Mixed disulfide} + \text{5-MNBA}$$

The rate of formation of the yellow color is followed spectrophotometrically at 410 nm and is a sensitive assay of the activity of serum cholinesterase.

The assay can be performed with and without dibucaine or fluoride for identification of cholinesterase variants (5,6).

REAGENT COMPOSITION

Reactive ingredients:

Butyrylthiocholine	7 mmol/L
5,5'-dithiobis-(2-nitrobenzoic acid)	0.25 mmol/L

Non-reactive ingredients:

Buffers, stabilizers and fillers

REAGENT PREPARATION

Dissolve the contents of each vial of Substrate and each vial of Starter with the volume of distilled or deionized water specified on the vial label.

REAGENT STORAGE AND STABILITY

The unreconstituted dry reagents are stable until the expiration date on the vial labels when stored at 2–8 °C.

Reconstituted stability:

Substrate:	Stable for 30 days at 2–8 °C. Store in amber bottle and protect from sunlight.
Starter:	Stable for 60 days at 2–8 °C. Store in amber bottle and protect from sunlight.

If the absorbance of the freshly reconstituted Substrate and Starter is greater than 0.700 when measured at 410 nm against a water blank, do not use; this indicates deterioration.

The reagent is light sensitive; avoid exposure to sunlight.

PRECAUTIONS

Good laboratory safety practices should be followed when handling any laboratory reagent. Refer to a recognized laboratory safety program for additional information. (See GP17-T, Clinical Laboratory Safety; Tentative Guideline (1994), National Committee on Clinical Laboratory Standards, Wayne, PA.)

Intended for in vitro diagnostic use only.

SPECIMEN COLLECTION, PREPARATION AND STORAGE

Serum, separated from the cells as soon as possible after collection, is the specimen of choice. Do not use hemolyzed samples.

Cholinesterase in serum is stable for 17 days at room temperature or refrigerated (4 °C) and for 3 months at -20 °C (8). If the samples show evidence of microbial contamination, do not use.

INTERFERING SUBSTANCES

Young (9) has published a comprehensive list of drugs and substances which cause changes in levels of cholinesterase or interfere with its determination.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Spectrophotometer or colorimeter capable of accurately measuring absorbance at 410 nm.
2. Matched cuvettes, preferably with 1 cm light path.
3. Constant temperature bath. If the assay is followed in the cuvette compartment of a spectrophotometer, this should be thermostated.
4. Distilled or deionized water.
5. Pipettes to measure water, reagent and samples.

MATERIAL PROVIDED

R85136: Cholinesterase, 20 × 6.5 mL

20 × 6.5 mL Substrate

5 × 5 mL Starter

TEST PROCEDURE

Bring needed volume of reagent and samples to incubation temperature. Then set up assay as follows:

Wavelength:	410 nm
Temperature:	30 °C
Cuvettes:	1 cm light path
Substrate:	3 mL
Starter:	0.1 mL

Mix and incubate at 30 °C. Then add:

Sample:	0.01 mL

Mix. Without delay read the change in absorbance in the spectrophotometer with the instrument adjusted to 0 absorbance with a water blank. Readings should be taken at 30 second intervals. Obtain the ΔA/30 seconds from the linear part of the assay.

QUALITY CONTROL

Serum controls are recommended to monitor the performance of manual and automated assay procedures, providing a continued screening of the instrument, reagents and techniques. Commercially available control material with established values for cholinesterase may be used. Assayed Control Serum, Level 1 (Cat. No. R83082) and Level 2 (Cat. No. R83083) are recommended for this purpose.

CALCULATIONS

$$\Delta A/30_S \times \frac{TV \times 1000}{13.5 \times LP \times SV \times t} = \text{U/L cholinesterase activity in sample}$$

Where:

TV	=	Total volume (3.11 mL)
13.5	=	Millimolar extinction coefficient of 5-MNBA
LP	=	Light path (1 cm)
SV	=	Serum volume (0.01 mL)
t	=	Time (0.5 min, since ΔA/30 sec. is used)

33057_01 11/10/09

1000 converts U/mL to U/L.

The conversion factor is:

$$\frac{3.11 \times 1000}{13.5 \times 1 \times 0.01 \times 0.5} = 46{,}074$$

Multiply the ΔA/30 sec. by this factor to obtain the U/L of cholinesterase activity in the sample.

Sample Calculation:

If the ΔA/30 was 0.186 then,

0.186 x 46074 = 8569 U/L cholinesterase activity in sample.

LIMITATIONS OF THE PROCEDURE

1. If the ΔA/30 seconds is greater than 0.250 repeat the assay using a sample diluted with 9 volumes of physiological saline (sodium chloride: 150 mmol/L in water). Multiply results by 10 to obtain the activity of the enzyme in the sample.
2. The reaction is very sensitive and fast. Do not delay taking readings after addition of sample to the mixed reagent.
3. We recommend the assay be performed at 30 °C. However, other temperatures, such as 25 °C or 37 °C can be employed in performing this assay. The enzyme activity will vary with changes in temperature, but the calculations will remain the same. The expected values, however, will be different.

REAGENT PERFORMANCE

1. Linearity: The assay is linear to 11,500 U/L serum cholinesterase activity.
2. Correlation: Results obtained using this reagent were compared to those obtained using a commercial reagent (Boehringer Mannheim Diagnostics) as a reference. Ninety-eight serum samples ranging in cholinesterase activity from 700 U/L to 13,500 U/L were assayed. The correlation coefficient was 0.998 and the regression equation was y = 1.058 x + 0.393.
3. Precision:

Within Run

Mean (U/L)	1300	4250	8220
SD	20	80	120
CV (%)	1.54	1.88	1.46
N	14	14	14

Run-to-Run

Mean (U/L)	1300	4250	8260
SD	20	50	130
CV (%)	1.54	1.18	1.57
N	14	14	14

REFERENCE RANGES

The following data was obtained at three different temperatures. Eighty-eight serum samples from apparently normal individuals were tested.

25 °C	30 °C	37 °C
1820 to 7580 U/L	2180 to 9180 U/L	2710 to 11510 U/L

It is recommended that each laboratory establish its own reference range.

REFERENCES

1. King, J., *Cholinesterase in Clinical Biochemistry*, Edited by H. Ch. Curtius and Marc Roth., W. DeGruyter, Berlin and New York, p.1161, 1974.
2. Silk, E., King. J. and Whittaker, M., *Annals of Clin. Biochem*. 16, 57, 1979.
3. *Textbook of Clinical Chemistry,* Edited by Norbert W. Tietz, W.B. Saunders Company, Third Edition, Philadelphia, p. 746, 1986.
4. Das, R.K. and Liddell, J., *J. Med. Genet*. 7, 351,1970.
5. Dietz, A.A., *Selected Methods of Clinical Chemistry*, Vol. 8, Amer. Assn. for Clin. Chemistry, Washington, D.C., p. 41, 1977.
6. Garry, P.J., *Clin. Chem*. 17, 183, 1971.
7. Ellman, G.L., et al., *Biochem. Pharmac*. 7, 88,1961.
8. *Clinical Chemistry: Principles and Technics*, Edited by R.J. Henry, Second Edition, Harper and Row, Publishers, Inc., New York, p. 917, 1974.
9. Young, D.S., *Effects of Drugs on Clinical Laboratory Tests*, *Third Edition*, AACC Press, Washington, D.C., 3.307-3.08, 1990.

IVD **For in vitro diagnostic use**

See package insert for proper use

CLINIQA CORPORATION
288 Distribution St.
San Marcos, CA 92078
USA

SALES AND TECHNICAL SUPPORT
P: 800 728 5205
+1 760 744 1900
F: +1 760 571 5198
www.cliniqa.com

FOR ORDERS AND CUSTOMER SERVICE
P: 800 728 5205
+1 760 744 1900
F: +1 760 571 5197
csgroup@cliniqa.com

EC REP
CEpartner4U
Esdoornlaan 13
3951 DB Maarn, The Netherlands
P: +31 (0)6 516 536 26

RE-ORDER INFORMATION
Cholinesterase Reagent

Catalog No.

REF R85136

Made in the USA

33057_01 11/10/09