Cholinesterase Reagent

Order No.  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 succinylcholine 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).

The assay of the enzyme cholinesterase in serum is clinically useful. This reagent is intended for in vitro measurement of the activity of cholinesterase in serum.

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. Thiocohline reacts with 5,5'-dithiobis-2-nitrobenzoic acid (Ellman's reagent: DTNB) (7) to form 5-mercaptopro-2-nitrobenzoic acid (5-MNBA) which has an intense yellow color.

The reaction is as follows:

\[
\text{Cholinesterase} \\
\text{Butyrylthiocholine} + H_2O \rightarrow \text{Butyrate} + \text{Thiocholine} \\
\text{Thiocholine} + \text{DTNB} \rightarrow \text{Mixed disulfide} + 5\text{-MNBA}
\]

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.

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.

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.

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

CALCULATIONS
\[
\Delta A/30\text{s} \times \frac{1000}{TV} = \text{U/L cholinesterase activity in sample}
\]

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 \(\Delta A/30\text{s}\) is used)

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 x 6.5 mL
20 x 6.5 mL Substrate
5 x 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 \(\Delta A/30\text{s}\) 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.

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1000 converts U/mL to U/L.
The conversion factor is:

\[
\frac{3.11 \times 1000}{1.35 \times 1 \times 0.01 \times 0.5} = 46,074
\]

Multiply the \( \Delta A/30 \) sec. by this factor to obtain the U/L of cholinesterase activity in the sample.

Sample Calculation:
If the \( \Delta A/30 \) was 0.186 then,

\[
0.186 \times 46074 = 8569 \text{ U/L cholinesterase activity in sample.}
\]

LIMITATIONS OF THE PROCEDURE
1. If the \( \Delta A/30 \) 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
   \[
   \begin{array}{ccc}
   \text{Mean (U/L)} & 1300 & 4250 & 8220 \\
   \text{SD} & 20 & 80 & 120 \\
   \text{CV} (%) & 1.54 & 1.88 & 1.46 \\
   \text{N} & 14 & 14 & 14 \\
   \end{array}
   \]
   Run-to-Run
   \[
   \begin{array}{ccc}
   \text{Mean (U/L)} & 1300 & 4250 & 8260 \\
   \text{SD} & 20 & 50 & 130 \\
   \text{CV} (%) & 1.54 & 1.18 & 1.57 \\
   \text{N} & 14 & 14 & 14 \\
   \end{array}
   \]

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

\[
\begin{array}{ccc}
25 \, ^\circ C & 1820 \text{ to } 7580 \text{ U/L} \\
30 \, ^\circ C & 2180 \text{ to } 9180 \text{ U/L} \\
37 \, ^\circ C & 2710 \text{ to } 11510 \text{ U/L} \\
\end{array}
\]

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

REFERENCES

RE-ORDER INFORMATION
Cholinesterase Reagent
Catalog No.

R85136

Made in the USA