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Product Information

Acetylcholinesterase Activity Assay Kit (Colorimetric)

Catalog Number **CS0003**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Acetylcholinesterase (AChE) is a serine hydrolase mainly found at neuromuscular junctions and cholinergic brain synapses.[1] AChE hydrolyzes choline esters. Its principal biological role is termination of impulse transmission at cholinergic synapses, by rapid hydrolysis of the neurotransmitter acetylcholine to acetate and choline.[1]

Organophosphorus poisons form covalent bonds with a serine residue at the active site of AChE, and are thus potent irreversible inhibitors of AChE.[2] AChE inhibitors are used in treatment of various neuromuscular disorders, [3] and have provided the first generation of drugs for the treatment of Alzheimer's disease. [4] AChE inhibitors prevent cholinesterase from breaking down acetylcholine, increasing both the level and duration of the neurotransmitter action.[5]

The Acetylcholinesterase Activity Assay Kit (Colorimetric) provides a simple, quick, and direct procedure for measuring AChE levels in a variety of samples such as serum and plasma. This assay is based on the Ellman method in which thiocholine, produced by AChE, reacts with 5,5′-dithiobis (2-nitrobenzoic acid) to form a colorimetric (412 nm) product, proportional to the AChE activity present.

This kit does not require weighing, or mixing of multiple reagents. The AChE activity is measured without the use of a calibration curve. The AChE Positive Control in the kit can be used to screen for AChE inhibitors.

This kit has a linear range of 10-600 units/liter of AChE activity.

Unit definition: One unit of AChE is the amount of enzyme that catalyzes the production of 1.0 μmole of thiocholine per minute at pH 8.0 at room temperature.

Components

This kit contains sufficient reagents for 200 tests.

Component	Component Number	Amount	Container Information
Assay Buffer	CS0003A	30 mL	Bottle
Substrate Mix*	CS0003B	2 vials	Amber glass vial
AChE Positive Control	CS0003C	50 μL	Plastic vial

* Light-sensitive: Protect from light.

Component Information

Assay Buffer (CS0003A): Ready-to-use. This component may be stored either at –20 °C or at 2–8 °C.

Substrate Mix (CS0003B): Lyophilized. Each vial is sufficient for 100 reactions in a 96 well plate. Note: The Substrate Mix is light-sensitive and should be stored protected from light.

AChE Positive Control (CS0003C): Supplied as a 50 μL solution.

Equipment Required, But Not Provided

- 96 well clear flat-bottom plates
- Spectrophotometric (412 ± 5 nm) multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Procedure

- Equilibrate all reagents to room temperature before use.
- **For convenience, an Excel-based calculation sheet is available on the Product Detail Page.** Use this sheet to calculate the amounts of reagents required, as well as to calculate the test results.

Sample preparation

In general, serum and plasma samples should be diluted 40 to 80-fold in Assay Buffer. However, it is recommended to test several dilutions to ensure the readings are within the linear range.

Tissue or cell lysates can be prepared by briefly sonicating or by homogenization in 0.1 M phosphate buffer, pH 8.0, followed by centrifugation at 14,000 rpm for 5 minutes. Use cleared supernatants for the assay. Best results are obtained when lysates are freshly prepared. If this is not feasible, lysates should be stored at 2–8 °C and used within 24 hours.

All samples should be in a final volume of 50 µL.

Note: Always include a sample blank, where the sample is replaced with 50 µL of Assay Buffer.

AChE Positive Control

If desired, the AChE Positive Control can be included, either as a positive control, and/or to screen for AChE inhibitors. Immediately prior to use, dilute an aliquot 500-fold in Assay Buffer, to a final volume of 50 µL per reaction. Unused material should be aliquoted and stored at –20 °C. Unused diluted material should be discarded. As a control, specific inhibitors of AChE can be included (e.g., Phenserine, Catalog Number P0111).

Substrate Mix preparation:

Reconstitute the Substrate Mix by adding 500 µL of ultrapure water to each vial and vortex. This reconstituted Substrate Mix is a 10× solution.
Note: Each vial is sufficient for 100 reactions in a 96 well plate.

Reconstituted unused material should be aliquoted and stored at –20 °C, for up to 1 month, protected from light. Immediately prior to use, dilute the 10× Substrate Mix solution 10-fold in Assay Buffer to a final volume of 50 µL per reaction. This is a 1× Substrate Mix.

Assay reaction:

1. Set the spectrophotometric multiwell plate reader to an absorbance at 412 ± 5 nm.
2. Add 50 µL of each sample to a well of a 96 well plate. If desired, include 50 µL of the diluted AChE Positive Control. Add 50 µL of Assay Buffer as a blank. Work in duplicate.
3. Initiate the reaction by adding 50 µL of the 1× Substrate Mix solution to the sample(s) and blank wells, for a final reaction volume of 100 µL/well.
4. Immediately read the absorbance in kinetic mode in 1 minute intervals for 5 minutes.

Note: This assay is based on an enzyme-catalyzed kinetic reaction. Addition of Substrate Mix should be quick. It is recommended to use orbital shaking for 10-15 seconds prior to the reading. If shaking is not available, briefly but thoroughly mix the reactions using a multichannel pipette.

Results

Calculations

1. **An Excel-based calculation sheet is available at the Product Detail Page.** Use this sheet to calculate the test results.
2. If the Excel-based calculation sheet at the Product Detail Page is not used, calculations should be performed as follows:
   i. Average the O.D. readings of each duplicate. Subtract the mean blank (no enzyme) of each time point from its respective mean sample time point. For example, the mean blank of the 1 minute reading should be subtracted from each mean sample reading of 1 minute.
   ii. Construct a kinetic curve, with time (in minutes) on the X-axis, and O.D. on the Y-axis. Calculate the linear regression equation. The slope is in units of O.D./minute.
   iii. To convert the data from O.D./minute to AChE activity in units, use the Beer-Lambert law:

$$A = \varepsilon \times l \times c$$

where:
A is the absorbance (in O.D.)

ε is the extinction coefficient (molar absorptivity constant), which, for TNB, is 13,600 liter/mol × cm

l is the path length, which, for a 100 µL solution in a well of a 96 well plate, is 0.3 cm

c is the AChE activity in units (µmole/minute)
Note: The reaction volume is 100 µL (1×10^{-4} liter).

Isolating the c variable, the Beer-Lambert equation can be rewritten as:

$$c = A / \varepsilon \times l$$

Adding units, the equation can be written as follows:

AChE activity = µmole/min (units) =

$$\frac{\text{Slope (OD/min)} \times 10^{-4}\text{ (liter)} \times 10^{6}\text{ (µmole/mole)}}{\text{13,600 (liter/(mole} \times \text{cm))} \times \text{0.3 cm}}$$

$$= \text{Slope} \times 0.0245$$

Therefore, to calculate the enzymatic activity in units, multiply the slope by 0.0245.

To calculate the enzymatic specific activity in units/liter, divide the enzymatic activity (in units) by the sample volume (50 µL, or 5×10^{-5} liter). If applicable, take into consideration the dilution factor.

References

1. Barnard, E.A., "Neuromuscular transmission - enzymatic destruction of acetylcholine", from *The Peripheral Nervous System* (Hubbard, J.I., ed.). Springer US / Plenum Press (New York, NY), pp. 201-224 (1974).
2. Quinn, D.M., *Chem. Rev.*, **87(5)**, 955-979 (1987).
3. Taylor, P., "Anticholinesterase agents", from *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 11th ed. (Brunton, L.L., *et al.*, eds.). McGraw-Hill (New York, NY), pp. 201-216 (2006).
4. Greenblatt, H.M. *et al.*, *J. Mol. Neurosci.*, **20(3)**, 369-383 (2003).
5. Čolović, M.B. *et al.*, *Curr. Neuropharmacol.*, **11(3)**, 315-335 (2013).

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