



Acetylcholinesterase Assay Kit

Catalog Number KA1607

100 assays

Version: 02

Intended for research use only

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Introduction

Intended Use

Application:

Direct assays of acetylcholinesterase activity in blood, serum, plasma, and other biological samples.
Evaluation of acetylcholinesterase inhibitors.

Features:

- ✓ Sensitive and accurate: Detection range 10 to 600 U/L AChE activity in 96-well plate assay.
- ✓ Convenient: The procedure involves adding a single working reagent, and reading the optical density at 2 min and 10 min at room temperature.
- ✓ High-throughput: Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

Principle of the Assay

ACETYLCHOLINESTERASE (EC 3.1.1.7, AChE), also known as RBC cholinesterase, is found primarily in the blood and neural synapses. Low serum cholinesterase activity may relate to exposure to insecticides or to one of a number of variant genotypes. AChE catalyzes the hydrolysis of the neurotransmitter acetylcholine into choline and acetic acid, a reaction necessary to allow a cholinergic neuron to return to its resting state after activation. Cholinesterase levels of cells and plasma are used as a guide in establishing safety precautions relative to exposure and contact, as well as a guide in determining the need for workers to be removed from areas of contact with the organic phosphate insecticides. Simple, direct and automation-ready procedures for measuring AChE activity are very desirable. Acetylcholinesterase Assay Kit is based on an improved Ellman method, in which thiocholine produced by the action of acetylcholinesterase forms a yellow color with 5,5'-dithiobis(2-nitrobenzoic acid). The intensity of the product color, measured at 412 nm, is proportionate to the enzyme activity in the sample.

General Information

Materials Supplied

List of component

Component	Amount
Assay Buffer: (pH 7.5)	30 mL
Reagent	240 mg
Calibrator: (equivalent to 200 U/L)	4 mL

Storage Instruction

Store all reagents at room temperature. Shelf life: 6 months.

Materials Required but Not Supplied

Pipeting (multi-channel) devices. Clear-bottom 96-well plates (e.g. Corning Costar) and plate reader.

Precautions for Use

- Precautions
- ✓ reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents.
- ✓ This assay is based on an enzyme-catalyzed kinetic reaction. Addition of Working Reagent should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.
- ✓ For assays in standard 1 mL cuvet, use 1 mL water and 1 mL Calibrator, 50 μ L sample + 950 μ L Working Reagent.

Assay Protocol

Reagent Preparation

The Working Reagent should be prepared freshly and used within 30 min. Each reaction well requires 2 mg reagent. Calculate the amount of reagent needed and weigh this amount (mg) in a centrifuge tube. Add 200 μ L Assay Buffer per 2 mg reagent. Vortex to dissolve.

Sample Preparation

Blood samples should be diluted 40-fold in the Assay Buffer, e.g. accurately pipet 5 μ L blood and mix thoroughly with 195 μ L Assay Buffer.

Tissue or cell lysates are prepared by brief sonication or homogenization in 0.1M phosphate buffer (pH 7.5), followed by centrifugation at 14,000 rpm for 5 min. Use supernatant for assay.

Ideally samples should be assayed fresh. If this is not possible, refrigerate samples and assay them within 24 hours.

Assay Procedure

- ✓ Calibrator: transfer 200 μ L water and 200 μ L calibrator separately into wells of a clear bottom 96-well plate. Samples: add 10 μ L sample per well in separate wells.
- ✓ Reaction: transfer 190 μ L freshly prepared Working Reagent to all sample wells and tap plate briefly to mix. Read OD_{412nm} at 2 min and at 10 min in a plate reader.

Data Analysis

Calculation of Results

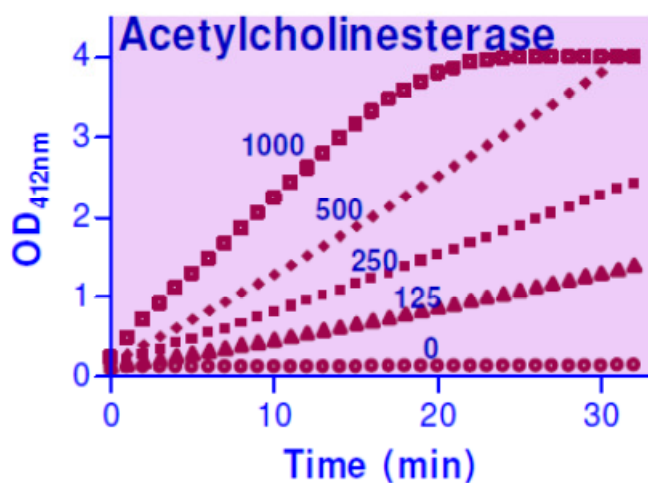
$$\text{AChE Activity} = \frac{\text{OD}_{10} - \text{OD}_2}{\text{OD}_{\text{CAL}} - \text{OD}_{\text{H}_2\text{O}}} \times n \times 200 (\text{U/L})$$

Where OD_{10} and OD_2 are the $\text{OD}_{412\text{nm}}$ values of the sample at 10 min and 2 min, respectively. OD_{CAL} and $\text{OD}_{\text{H}_2\text{O}}$ are the $\text{OD}_{412\text{nm}}$ values of the Calibrator and water at 10 min. n is the dilution factor ($n = 40$ for whole blood). The number “200” is the equivalent activity of the calibrator under the assay conditions.

Note: if the AChE activity without consideration of the dilution factor is higher than 600 U/L, dilute sample further in Assay Buffer and repeat the assay. Multiply the results by the dilution factor.

Unit definition: one unit of enzyme catalyzes the production of 1 μmole of thiocholine per minute under the assay conditions (pH 7.5 and room temperature).

Two human blood samples were assayed in duplicate using the 96-well plate protocol. The AChE activities were $3,402 \pm 163$ and $3,660 \pm 151$ U/L.



Kinetics of 0-1000 U/L Acetylcholinesterase Reaction in 96-well plate

Resources

References

1. Magnottl, RA. et al. (1987). Measurement of Acetylcholinesterase in Erythrocytes in the Field. Clin. Chem. 33/10, 1731-1 735.
2. Kovarik, Z et al. (2003). Acetylcholinesterase active centre and gorge conformations analysed by combinatorial mutations and enantiomeric phosphonates. Biochem. J. (2003) 373, 33–40.
3. Ordentlich, A. et al. (1996). The Architecture of Human Acetylcholinesterase Active Center Probed by Interactions with Selected Organophosphate Inhibitors. J. Biol. Chem. 271 (20): 11953–11962.