Acetylcholinesterase Reagent (DACEN25mL)

Rapid Colorimetric Determination of Acetylcholinesterase Activity

DESCRIPTION

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. BioAssay Systems' acetylcholinesterase reagent 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 at 410 nm is proportionate to the enzyme activity in the sample.

APPLICATIONS

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

KEY FEATURES

Simple and Convenient. This single reagent is ready to use, when reconstituted in 25mL deionized water.
High-throughput. Can be readily adapted on chemistry analyzers or high-throughput liquid handling system to test thousands of samples per day.

KIT CONTENTS

Reagent: powder in one 30mL amber bottle.
Catalog#: DACEN25ML. Sufficient for approximately 100 tests.
Bulk reagent (>5,000 tests) are available upon request.

Storage conditions. The reagent is shipped at room temperature. Store unopened at 2-25°C in a dry environment. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Working Reagent: Before using the Reagent, lightly tap it to settle powder. Add the 25 mL distilled or deionized water. Cap the bottle, thoroughly mix to dissolve content by shaking the bottle for 10 seconds. Reconstituted Working Reagent is a pale yellowish liquid, pH 7.2. Prepared Reagent is stable for 8 hours at room temperature or 3 days at 2-8°C, protected from light.

Samples: Whole blood, serum or EDTA plasma (use of anticoagulants other than EDTA is not recommended). Avoid hemolysis. If particles are present in sample, centrifuge at 14,000 rpm for 5 min and use supernatant. For Cholinesterase, whole blood and plasma samples are stable 14 days at room temperature or when refrigerated at 2 - 8ºC.

Calibrators or Standards: Calibrators or standards are required but not supplied. They are commercially available, e.g. Roche Diagnostics PreciNorm U Plus Control Serum Chemistry Normal 10 x 3mL (Fisher Scientific Catalog No. 50-929-48).

Assays on Chemistry Analyzers: For optimal performance of the assay, follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions. The performance of the assays must be validated and defined by the user.

Reference settings for Beckman Coulter AU680
- Sample: 2.0 uL
- Reagent: 225 uL
- Wavelength: 410nm (or 405nm), sec. 660 nm
- Method: Rate

96-Well Plate Assay Procedure: 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. Detection range: 6 – 750 U/L.

1. *Calibrator/Standard:* transfer 5 µL calibrator separately into wells of a clear bottom 96-well plate.

   Samples: transfer 5 µL sample per well in separate wells.

2. *Reaction*: transfer 225 µL freshly prepared Working Reagent to all assay wells and tap plate briefly to mix.

   Read OD_{410nm} at 1 min and at 10 min in a plate reader. Alternatively, record kinetics at 410 nm.

3. *Calculation:* acetylcholinesterase activity is calculated as follows,

$$\text{ACHE Activity} = \frac{(OD_{10} - OD_1)_{SAMPLE}}{(OD_{10} - OD_1)_{CAL}} \times \text{Cal (U/L)}$$

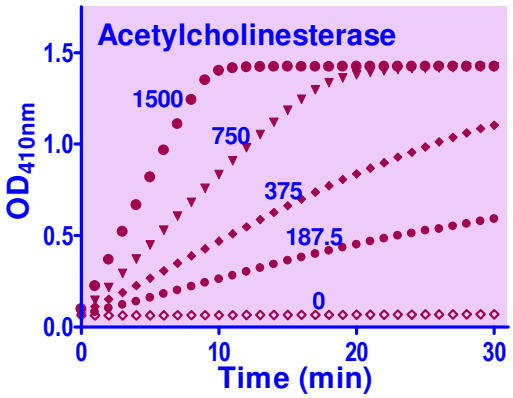
   Where OD_{10} and OD_1 are the OD_{410nm} values at 10 min and 1 min, respectively. Cal (U/L) is the ACHE activity of the calibrator used.

Note: If the calculated AChE activity is higher than 750 U/L, dilute sample in 0.9% NaCl 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 7.2 and room temperature).

MATERIALS REQUIRED, BUT NOT PROVIDED

Calibrators/standards: e.g. Roche Diagnostics PreciNorm U Plus Control Serum Chemistry Normal 10 x 3mL, Catalog No. 50-929-48 (Fisher Scientific). 0.9% NaCl solution. Distilled or deionzed water.
Equipment: chemical analyzers capable of kinetic rate determination at 405nm or 410nm. Pipeting (multi-channel) devices. 96-Well Plate Procedure: Clear-bottom 96-well plates (e.g. Corning Costar) and plate reader.



Kinetics of 0-1500 U/L Acetylcholinesterase Reaction in 96-well plate

Literature

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.