

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 catalyses 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 organic phosphate insecticides. This reagent is based on an improved Ellman method, in which thiocholine produced by the action of acetylcholinesterase forms a yellow colour with 5,5'-dithiobis(2-nitrobenzoic acid). The intensity of the product colour at 410 nm is proportional to the enzyme activity in the sample.

Specifications

Detection Method	Colorimetric (OD 410 nm; improved Ellman method)
Detection Range	6 - 750 U/L
Sample Type	Whole blood, serum, EDTA plasma and other biological samples
Species Reactivity	All
Assay Time	10 minutes (kinetic read at 1 min and 10 min)
Kit Size	100 Assays
Equipment Required	Microplate reader
Storage	Shipped at room temperature. Store unopened at 2-25 degrees C in a dry environment.
Shelf Life	6 months after receipt
Shipping	Room Temperature

Kit Components

Component	Quantity	Storage
Reagent (powder in one 30 mL amber bottle)	sufficient for approximately 100 tests	2-25 degrees C, dry

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0208	100 Assays

Assay Procedure

Note: This is a sample protocol. Protocols are batch/lot specific - always follow the instructions supplied with your kit.

1. Before using the reagent, lightly tap it to settle the powder, add 25 mL of distilled or deionised water, cap the bottle and thoroughly mix to dissolve the contents by shaking for 10 seconds; the reconstituted Working Reagent is a pale yellowish liquid, pH 7.2.
2. Prepared reagent is stable for 8 hours at room temperature or 3 days at 2-8 degrees C, protected from light.
3. Transfer 5 uL of calibrator into separate wells of a clear-bottom 96-well plate.
4. Transfer 5 uL of each sample per well into separate wells.
5. Transfer 225 uL of freshly prepared Working Reagent to all assay wells and tap the plate briefly to mix.

6. Read OD410nm at 1 minute and at 10 minutes in a plate reader, or alternatively record kinetics at 410 nm.

Calculation

Acetylcholinesterase activity is calculated as: $\text{ACHE Activity} = \frac{(\text{OD}_{10} - \text{OD}_1)_{\text{SAMPLE}}}{(\text{OD}_{10} - \text{OD}_1)_{\text{CAL}}} \times \text{Cal (U/L)}$, where OD10 and OD1 are the OD410nm values at 10 minutes and 1 minute respectively, and Cal (U/L) is the AChE activity of the calibrator used. If the calculated AChE activity is higher than 750 U/L, dilute the sample in 0.9% NaCl, repeat the assay and multiply the result by the dilution factor. Unit definition: one unit of enzyme catalyses the production of 1 umole of thiocholine per minute under the assay conditions (pH 7.2 and room temperature).

Notes

- Samples: whole blood, serum or EDTA plasma (use of anticoagulants other than EDTA is not recommended). Avoid haemolysis. If particles are present, centrifuge at 14,000 rpm for 5 minutes and use the supernatant. Whole blood and plasma samples are stable for 14 days at room temperature or when refrigerated at 2-8 degrees C.
- Calibrators or standards are required but not supplied; they are commercially available.
- For assays on chemistry analysers, follow the directions for the analyser concerned and refer to the appropriate operator's manual; performance must be validated and defined by the user. Reference settings for a Beckman Coulter AU680: sample 2.0 uL, reagent 225 uL, wavelength 410 nm (or 405 nm) with secondary 660 nm, method Rate.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.

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Technical specifications are based on manufacturer documentation and may be updated. Protocols shown are summaries - always follow the instructions supplied with your kit/lot.