

Product Information

Acetylcholinesterase Activity Assay Kit

Catalog Number **MAK119**
Storage at Room Temperature

TECHNICAL BULLETIN

Product Description

Acetylcholinesterases (AChEs) are enzymes that hydrolyze the neurotransmitter acetylcholine (ACh) to acetate and choline. AChE is located at the synaptic cleft and functions to terminate synaptic transmission by catalyzing the breakdown of ACh allowing cholinergic neurons to return to a resting state after activation. Changes in AChE activity may result from exposure to certain insecticides, which act as cholinesterase inhibitors. Inhibitors of AChE are also used to treat certain conditions such as dementia.

The Acetylcholinesterase Activity Assay kit provides a simple and direct procedure for measuring AChE levels in a variety of samples such as blood, serum, and plasma. This assay is an optimized version of the Ellman method in which thiocholine, produced by AChE, reacts with 5,5'-dithiobis(2-nitrobenzoic acid) to form an colorimetric (412 nm) product, proportional to the AChE activity present. One unit of AChE is the amount of enzyme that catalyzes the production of 1.0 μ mole of thiocholine per minute at pH 7.5 at room temperature. This kit has a linear range of 10–600 units/L of AChE activity.

Components

The kit is sufficient for 100 assays in 96 well plates.

Assay Buffer, pH 7.5 Catalog Number MAK119A	30 mL
Calibrator, equivalent to 200 U/L Catalog Number MAK119B	4 mL
Reagent Catalog Number MAK119C	240 mg

Reagents and Equipment Required but Not Provided.

- Spectrophotometric multiwell plate reader
- Clear 96 well flat-bottom plate

Precautions and Disclaimer

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

Storage/Stability

This kit is shipped, and should be stored at room temperature.

Procedure

Sample Preparation

Blood samples should be diluted 40-fold in Assay Buffer.

Tissue or cell lysates can be prepared by briefly sonicating or by homogenization in 0.1 M phosphate buffer, pH 7.5, followed by centrifugation at 14,000 rpm for 5 minutes. Used cleared supernatants for 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.

Assay Reaction

1. The Working Reagent should be prepared fresh and used within 30 minutes. Each reaction will require 2 mg of Reagent. To prepare Working Reagent, calculate the amount of reagent needed in total and weigh that amount into a centrifuge tube. Add 200 μ L of Assay Buffer per 2 mg of Reaction and vortex to dissolve.
2. Transfer 200 μ L of water (Assay Blank) and 200 μ L of Calibrator into separate wells of a 96 well plate. Add 10 μ L of samples into separate wells of the 96-well plate.
3. Transfer 190 μ L of the freshly prepared Working Reagent to all sample wells and tap plate briefly to mix.
4. Incubate the samples at room temperature. After 2 minutes, take the initial absorbance measurement at 412 nm $(A_{412})_{\text{initial}}$.
5. Continue to incubate the plate at room temperature. At 10 minutes, take the final measurement $(A_{412})_{\text{final}}$.

Note: 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 multichannel pipette is recommended.

These assays can be adapted for use in standard 1 mL cuvettes. To adapt to cuvettes follow procedure as for 96 well plates but change volumes as follows: 1 mL of water, 1 mL of Calibrator, 50 μ L of sample, and 950 μ L of Working Reagent.

Calculations

AChE Activity (units/L) =

$$\frac{(A_{412})_{\text{final}} - (A_{412})_{\text{initial}}}{(A_{412})_{\text{calibrator}} - (A_{412})_{\text{blank}}} \times n \times 200$$

200 = equivalent activity (units/L) of the Calibrator when assayed is read at 2 minutes and 10 minutes

n = dilution factor (n = 40 for whole blood)

$(A_{412})_{\text{calibrator}}$ = Absorbance of the calibrator at 10 minutes

$(A_{412})_{\text{blank}}$ = Absorbance of the blank at 10 minutes

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

One unit of AChE is the amount of enzyme that catalyzes the production of 1.0 μ mole of thiocholine per minute at room temperature at pH 7.5. This kit has a linear range of 10–600 units/L of AChE activity.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For UV assays, use clear plates that are UV transparent or quartz plates.
Samples with erratic readings	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
	Samples measured at incorrect wavelength	Check the equipment and filter settings

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