Acetylcholinesterase (AchE) Assay Kit

Catalog No.: abx298834

Size: 96 tests (96 samples)

Detection Range: 1.225 U/ml – 490 U/ml

Sensitivity: 1.225 U/ml

Storage: Store all components at 2 – 8°C for up to 6 months.

Application: For detection and quantification of AChE activity in serum, plasma, tissue, and other biological fluids.

Introduction

Acetylcholinesterases (AChEs) are enzymes that hydrolyze acetylcholine (ACh), a neurotransmitter, to acetic acid 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.

Abbexa's Acetylcholinesterases (AChEs) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating AChE activity. Tricholine, produced by AChE reacts with DTNB to form a colorimetric product (TNB), which produces an absorbance at 412 nm. The absorbance should be measured at 412 nm. The intensity of the color is proportional to the activity of the AChE, which can then be calculated.

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Kit components

1. 96-well microplate
2. Lysis buffer: 2 × 50 ml
3. Buffer solution: 30 ml
4. Chromogenic agent: 1 vial
5. Substrate: 1 vial
6. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (412 nm)
2. Double distilled water
3. Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. Vials/tubes
6. Incubator or Sonicating water bath
7. Centrifuge
8. Vortex mixer

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Plasma:** Collect plasma using heparin as the anticoagulant. Centrifuge for 10 mins at 1000-2000 x g at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue Homogenates:** Weigh the tissue homogenate. For each 1 g of homogenate, add 9 ml lysis buffer. Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 10,000 x g at 4°C for 10 min. Collect the supernatant and assay immediately. The protein concentration in the supernatant should be determined separately.

Samples should not contain detergents such as SDS Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4), then carry out the assay procedure.

The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Mouse Serum	8-20
Mouse Plasma	4-10
Human Serum	4-10
Human Plasma	4-10
Rat Serum	4-10
Dog Serum	4-10
Horse Serum	2-8
10% Mouse Liver Tissue Homogenate	1
10% Mouse Kidney Tissue Homogenate	1
10% Mouse Brain Tissue Homogenate	2-8
10% Crucian carp muscle Tissue Homogenate	1

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Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Chromogenic agent working solution:** Dissolve a vial of Chromogenic agent with 22 ml of Buffer solution. Prepare immediately before carrying out the assay. Unused Enzyme Chromogenic agent working solution can be stored in dark at 2-8°C for up to 7 days.
- **Substrate working solution:** Dissolve a vial of Substrate with 1.3 ml of Buffer solution. Prepare immediately before carrying out the assay. Unused Enzyme Chromogenic agent working solution can be stored in dark at 2-8°C for up to 7 days.

B. Assay Procedure

1.1. Set the sample wells and add 20 µl of sample to each Sample well.
1.2. Add 170 µl of Chromogenic agent working solution to each well.
1.3. Add 10 µl of Substrate working solution to each well.
1.4. Gently tap the plate to mix, or use a microplate shaker, measure the OD of each well with a microplate reader at 412 nm within 5 minutes.
1.5. Measure and record OD at 30 seconds and 330 seconds as A1 and A2 respectively.

C. Calculation of Results

1. Serum and plasma samples:

One unit of AChE activity is defined as the quantity of AChE in 1 ml of sample that catalyzes the production of 1 nmol TNB in a minute.

$$\text{AChE activity (U/ml)} = \frac{\left(\Delta A \times \frac{V_{Total}}{\varepsilon \times d} \times 10^9\right)}{\frac{V_{Sample}}{T}} \times f$$

$$= 245 \times \Delta A \times f$$

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2. Tissues samples:

AChE activity per mg of protein: One unit of AChE activity is defined as the quantity of AChE in 1 mg of protein that catalyzes the production of 1 nmol TNB in a minute.

$$\text{AChE activity (U/mg protein)} = \frac{\left(\Delta A \times \frac{V_{Total}}{\varepsilon \times d} \times 10^9\right)}{\frac{C_p \times V_{Sample}}{T}} \times f$$

$$= \frac{245 \times \Delta A}{C_P \times f}$$

AChE activity per mg of tissue weight: One unit of AChE activity is defined as the quantity of AChE in 1 mg of tissue sample that catalyzes the production of 1 nmol TNB in a minute.

$$\text{AChE activity (U/mg tissue weight)} = \frac{\left(\Delta A_{412} \times \frac{V_{Total}}{\varepsilon \times d} \times 10^9\right)}{\frac{\frac{W \times V_{Sample}}{V_{Total\ sample}}}{T}} \times f$$

$$= \frac{245 \times \Delta A}{W \times f}$$

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where:

ΔA_{412}	OD value of the sample $(OD_{at\ 330\ seconds} - OD_{at\ 30\ seconds})$
ε	molar extinction coefficient of TNB, 13.6×10^4 L/mol/cm
d	optical path of 96 well microplate, 0.6 cm
a	gradient of the standard curve (linear fit)
b	y-intercept of the standard curve (linear fit)
V_{Total}	total volume of reaction system, 2×10^{-4} L
V_{Sample}	volume of sample added into the reaction system, 2×10^{-2} ml
$V_{Total\ sample}$	volume of added extraction solution, 1ml
10^9	unit conversion, 1 mol=10^9 nmol
T	reaction time, 5 min
W	weight of sample (g)
C_P	concentration of protein in sample (mg protein/ml)
f	dilution factor of the sample before carrying out the assay