ACHE Activity Assay Kit (Colorimetric)

Catalog Number KA3962
100 assays
Version: 02

Intended for research use only
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Introduction

Intended Use

- Application:
  ✓ Measurement of AChE activity in various tissues/cells
  ✓ Screening of AChE inhibitors

- Sample Type
  ✓ Serum, plasma or blood.
  ✓ Animal tissues: liver, heart, kidney, etc.
  ✓ Cell culture: Adherent or suspension cells

Background

Acetylcholinesterase (AChE) is one of the most crucial enzymes associated with nerve response and function. AChE is a serine protease that hydrolyzes the neurotransmitter acetylcholine. Irreversible inhibition of AChE may lead to muscular paralysis, convulsions, bronchial constriction, and death by asphyxiation. ACHE Activity Assay Kit (Colorimetric) provides a simple and sensitive method for continuous monitoring of enzymatic activity using colorimetry (OD 570 nm). In this assay, AChE converts acetylcholine substrate to choline, which is then oxidized by choline oxidase (CO) to produce an intermediate. The intermediate reacts with a highly specific probe to generate color. This assay kit can detect acetylcholinesterase activity as low as 0.5 mU/ml in a variety of samples.
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>AChE Assay Buffer</td>
<td>25 ml</td>
</tr>
<tr>
<td>AChE Probe (DMSO)</td>
<td>200 µl</td>
</tr>
<tr>
<td>AChE Substrate (Lyophilized)</td>
<td>1 vial</td>
</tr>
<tr>
<td>Choline Oxidase Enzyme Mix (Lyophilized)</td>
<td>1 vial</td>
</tr>
<tr>
<td>Choline Standard (Lyophilized)</td>
<td>1 vial</td>
</tr>
<tr>
<td>AChE Positive Control (Lyophilized)</td>
<td>1 vial</td>
</tr>
</tbody>
</table>

Storage Instruction

Store kit at -20°C, protected from light. Warm Assay Buffer to room temperature before use. Read the entire protocol before performing the assay.

Materials Required but Not Supplied

✓ 96-well clear plate with flat bottom
✓ Multi-well spectrophotometer

Precautions for Use

FOR RESEARCH USE ONLY! Not to be used on humans
Assay Protocol

Reagent Preparation

- AChE Probe: Store at -20°C. Warm to room temperature before use. Use within two months.
- AChE Substrate: Reconstitute with 110 µl AChE Assay Buffer to generate 50 mM solution. Store at -20°C. Use within two months. Keep on ice while in use.
- Choline Standard: Reconstitute with 100 µl AChE Assay Buffer to generate 50 mM stock Choline Standard Solution. Use within 2 months.
- AChE Positive Control: Reconstitute with 100 µl of AChE Assay Buffer. Aliquot and store at -20°C. Use within two months.

Assay Procedure

1. Sample Preparation: Homogenize 25 mg of sample (wet weight or cell pellet) in extraction buffer of your choice. Centrifuge at 10,000 X g for 5 min. Collect the supernatant. Add 1-5 µl of supernatant into a 96-well plate and adjust the volume to 50 µl with AChE Assay Buffer. Serum, plasma or blood (1-5 µl) can be tested directly.

   Notes:
   ✓ For unknown samples, we suggest testing several doses to ensure the reading are within the Standard Curve range.
   ✓ For samples having background, prepare parallel well(s) containing the same amount of sample as in the test well. Adjust the volume to 50 µl with AChE Assay Buffer.
   ✓ Extraction buffers containing 1% or more of Triton X-100 will interfere with the assay.

2. Standard Curve Preparation: Dilute Choline Standard to 0.5 mM by adding 10 µl of 50 mM Choline Standard into 990 µl of AChE Assay Buffer, mix well. Add 0, 2, 4, 6, 8 and 10 µl of the diluted 0.5 mM Choline Standard into a series of wells in 96-well plate. Adjust the volume to 50 µl/well with the AChE Assay Buffer to generate 0, 1, 2, 3, 4 and 5 nmol/well of Choline Standard.

3. AChE Positive Control: Dilute positive control solution 1:10 in AChE Assay Buffer. Add 1-10 µl of diluted Positive Control into desired wells(s) and adjust the final volume to 50 µl with AChE Assay Buffer.

4. Reaction Mix: Mix enough reagents for the number of assays (samples, Standards & Positive Control) to be performed. For each well, prepare 50 µl Reaction Mix containing:
<table>
<thead>
<tr>
<th></th>
<th>Reaction Mix</th>
<th>Background Control Mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>AChE Assay Buffer</td>
<td>45 µl</td>
<td>46 µl</td>
</tr>
<tr>
<td>Choline Oxidase Enzyme Mix</td>
<td>2 µl</td>
<td>2 µl</td>
</tr>
<tr>
<td>AChE Probe</td>
<td>2 µl</td>
<td>2 µl</td>
</tr>
<tr>
<td>AChE Substrate</td>
<td>1 µl</td>
<td>--</td>
</tr>
</tbody>
</table>

Mix well. Add 50 µl of Reaction Mix to each well containing the Choline Standards, Positive Control and samples. Mix well.

* For samples having high background, add 50 µl of Background Control Mix to sample background control well(s). Mix well.


*Note: Incubation time depends on the AChE Activity in the samples. We recommend measuring OD in a kinetic mode, and choosing two time points (T₁ and T₂) in the linear range to calculate the AChE activity of the samples. The Standard Curve can be read in end point mode (i.e. at the end of incubation time).*
Data Analysis

Calculation of Results

Subtract 0 Choline Standard reading from all readings. Plot the Choline Standard Curve. If sample background control reading is significant, subtract background control reading from sample readings. Calculate the AChE activity of the test sample: $\Delta \text{OD} = A_2 - A_1$. Apply the $\Delta \text{OD}$ to the Choline Standard Curve to get B nmol of Choline generated by AChE during the reaction time ($\Delta T = T_2 - T_1$).

\[
\text{Sample AChE Activity} = \frac{B}{(\Delta T \times V)} \times D = \text{nmol/min/ml} = \text{mU/ml}
\]

Where:
- B is the Choline amount from the Standard Curve (nmol)
- $\Delta T$ is the reaction time (min.)
- V is the sample volume added into the reaction well (ml)
- D is the sample dilution factor

Unit Definition: One milliunit of AChE activity is the amount of enzyme that will generate 1.0 nmol of Choline per min. at pH 7.4 at 37°C.

(a) ![Choline Standard Curve](image)

(b) ![Graph of OD 570nm vs Time](image)

Figure: Choline Standard Curve (a). AChE activity in serum (1 µl) & Positive Control (1 µl). Assays were performed following the kit protocol.