(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Acetylcholinesterase (A-CHE) Assay Kit

Catalog No.: BC00204

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Acetylcholinesterase (A-CHE) Assay Kit
Detection Method	Colorimetric
Sample Type	Tissue, serum, plasma, whole blood
Assay Type	Quantitative
Detection Instrument	Spectrophotometer or Microplate reader (412nm)

Principle

Acetylcholinesterase hydrolyzes acetylcholine to produce choline and acetic acid. Choline reacts with sulfhydryl chromogenic reagent to form yellow Sym-Trinitrobenzene (TNB). Colorimetric quantification is performed according to color intensity, and the content of hydrolyzed choline reflects the activity of cholinesterase.

Components

No.	Components	Size	Notes
Reagent 1	Standard powder	3 vials	Store at 4°C. 1 μmol/mL Standard Working solution preparation: Dissolve one vial of standard substance in 10 mL normal saline and mix well. Prepare freshly before use.
Reagent 2	Substrate	2 vials	Store at 4°C. Preparation of Substrate Buffer: Add 20 mL normal saline to each vial of powder before use to prepare substrate buffer, which can be stored at 4°C for two weeks.

Reagent 3	Chromogenic	3 mL	Store at 4°C. Preparation of Chromogenic Working Solution: Dilute the chromogenic stock solution with normal saline at a ratio of 1:9 before use to prepare the chromogenic working solution. Prepare as needed, or prepare 30 mL at a time. Store away from light at 2-8 °C for up to 3 months.
Reagent 4	Inhibitor	2 mL	Store at room temperature. Transfer it into an empty small plastic bottle after opening.
Reagent 5	Clearing agent	6 mL	Store at room temperature. Precipitation or turbidity may occur in cold weather; heat at 37°C until clear before use.
Reagent 6	Normal saline	60 mL×2	Store at room temperature.
Consumable 1	Microplate	1 plate	RT
Consumable 2	Plate Sealer	2 pieces	RT

Storage

The validity period of the kit is 6 months.

Preparation

Spectrophotometer equipped with 412 nm wavelength and 0.5 cm path length cuvettes (or microplate reader), 37°C water bath or constant temperature incubator, desktop low-speed centrifuge, pipettes of various specifications, double distilled water, vortex mixer, test tubes or centrifuge tubes, protein assay reagents (for tissue sample, available from our company).

- **Sample handling**

1. Tissue Samples: Accurately weigh the tissue sample. Add 9 times the volume of normal saline at a weight (g) to volume (mL) ratio of 1:9. Perform mechanical homogenization in

an ice-water bath, then centrifuge at 2500 rpm for 10 minutes. Collect the supernatant for subsequent detection. Meanwhile, take part of the supernatant to determine protein concentration. Protein quantification kits are available from our company.

2. Serum (Plasma): Take non-anticoagulated or anticoagulated whole blood, centrifuge at 1000-1500 rpm for 8 minutes, and collect the upper serum or plasma. For routine detection, dilute serum or plasma with normal saline at a ratio of 1:9 (the specific dilution ratio shall be subject to pre-experimental results).
3. Whole Blood Sample: Take 0.1 mL anticoagulated whole blood and add double distilled water to make up to 10 mL for 1:99 dilution, then mix thoroughly. Reduce the sampling volume if the sample quantity is limited. For instance, take 0.01 mL or 0.02 mL anticoagulated whole blood and dilute to 1 mL or 2 mL with double distilled water. Take a mL (typically 0.1 mL) for detection. Fully mix each sample thoroughly before sampling.

Operation process

	Assay tube	Control tube	Standard tube	Blank tube
Sample (mL)	a*			
1 μmol/mL Standard Working solution (mL)			a*	
Double distilled water (mL)				a*
Substrate Buffer (mL)	0.5	0.5	0.5	0.5
Chromogenic Working Solution (mL)	0.5	0.5	0.5	0.5
Mix well and incubate accurately at 37°C for 6 minutes.				
Inhibitor (mL)	0.03	0.03	0.03	0.03
Clearing agent (mL)	0.1	0.1	0.1	0.1
Sample (mL)		a*		
Mix well and stand at room temperature for 15 minutes. Set zero with double distilled water, measure the absorbance (A) of each tube at wavelength 412 nm with 0.5 cm optical path.				

Notes:

1. Control tubes must be prepared for every sample, as there are significant differences in absorbance among control tubes of different samples.
2. Let the test tubes stand at room temperature for 15 minutes before colorimetry. Precipitation or turbidity may occur at excessively low room temperature. In this case, place the tubes in a 37°C water bath for a short while until clarified. Colorimetry can be performed afterwards, which has no impact on experimental results.
3. Given the short reaction time, do not test excessive samples in one batch during batch detection. Strictly control the reaction time; otherwise, the experimental accuracy will be affected.
4. The volume of sample, standard substance and double distilled water marked as a* shall be equal:
   ① Dilute serum/plasma 10-fold with normal saline before detection, with a recommended sampling volume of 30~50 μL.
   ② The recommended sampling volume of 10% brain tissue homogenate is 30~50 μL.
   ③ Take 0.1 mL of whole blood diluted at the ratio of 1:99, and shake each tube well before sampling.

Calculation

1. Calculation of Acetylcholinesterase in Tissue Homogenate

   Definition: One unit of enzyme activity is defined as the amount of acetylcholinesterase that hydrolyzes 1 μmol substrate in the reaction system per milligram of tissue protein after incubation at 37°C for 6 minutes.

$$\text{A} - \text{CHE Activity (U/mg)} = \frac{\text{A}_{\text{Assay}} - \text{A}_{\text{Control}}}{\text{A}_{\text{Standard}} - \text{A}_{\text{Blank}}} \times \text{C}_{\text{Standard}} \div \text{Cpr}$$

2. Calculation of Acetylcholinesterase in Serum(plasma)

   Definition: One activity unit is defined as the amount of enzyme that hydrolyzes 1 μmol substrate in the reaction system per milliliter of serum sample after incubation at 37°C for 6 minutes.

$$\text{A} - \text{CHE Activity (U/mL)} = \frac{\text{A}_{\text{Assay}} - \text{A}_{\text{Control}}}{\text{A}_{\text{Standard}} - \text{A}_{\text{Blank}}} \times \text{C}_{\text{Standard}} \times \text{N}$$

3. Calculation of Acetylcholinesterase in Whole Blood

   Definition: One activity unit is defined as the enzyme quantity that hydrolyzes 1 μmol substrate in the reaction system per milliliter of whole blood after incubation at 37°C for 6 minutes.

$$\text{A} - \text{CHE Activity (U/mL)} = \frac{\text{A}_{\text{Assay}} - \text{A}_{\text{Control}}}{\text{A}_{\text{Standard}} - \text{A}_{\text{Blank}}} \times \text{C}_{\text{Standard}} \times \text{N}$$

Annotation:

$\text{C}_{\text{Standard}}$: Standard solution concentration, 1μmol/mL

Cpr: Protein concentration of homogenate, mg/mL

N: Dilution multiple before sample testing

Precautions

1. It is recommended to use disposable laboratory test tubes for operation.
2. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.