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Ver.250501

## **Acetylcholinesterase (AChE) Assay Kit**

BC8801-01(50 Tests/ 24 Samples)

**FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS**

## Product Description

AchE catalyzes the hydrolysis of acetylcholine to form choline, and choline react with dithio p-nitrobenzoic acid (DTNB) to form 5-mercapto-nitrobenzoic acid (TNB). TNB has an absorption peak at 412 nm. And the activity of AchE is calculated by measuring the increasing rate of absorbance at 412 nm.

## Kit components

Reagent	Volume	Storage
Extraction Solution	30mL	2-8°C
Reagent I	50mL	2-8°C
Reagent II	Powder × 2	2-8°C
Add 2.6mL Reagent I before use and dissolve completely. The unused reagent shall be made into aliquotes and stored at 2-8°C for 1 week.		
Reagent III	6mL	2-8°C
Reagent IV	6mL	2-8°C

## Reagents and Equipment Required but Not Provided

Refrigerated Centrifuge, Water Bath, Spectrophotometer, 1mL Glass Cuvette, Pipette Gun, Mortar/ Homogenizer and Distilled Water, Ultrasonicator.

## Protocol

### I. Enzyme extraction:

1. Tissues: To 0.1 g tissues and add 1mL extract solution. Keep on the ice bath to homogenate. Centrifuge at 8000 g, 4°C for 10 minutes, take the supernatant for the assay.
2. Bacteria and cells: Add 1mL of Extract solution to 5 million cells. Ultrasonicate (power 300W, ultrasonic 3seconds, interval 7seconds, total time 3minutes) while keeping the tube on ice; Then Centrifuge at 8000 g, 4°C for 10 minutes, take the supernatant, keep on ice for till the assay is performed.
3. Serum and other liquids: Direct determination.

### II. Determination procedure:

1. Preheat the spectrophotometer for 30 minutes, adjust the wavelength to 412 nm and set the counter to zero with distilled water.
2. Operation table:

Reagent(μL)	Test tube (T)	Control tube (C)
Sample	30μL	30μL
Reagent II	100μL	100μL
Incubate at 37°C for 5 minutes		
Reagent IV	100μL	100μL
Reagent II	-	100μL

3. Mix thoroughly, centrifuge at 12000 rpm for 5 minutes.
4. Collect the supernatant and proceed as mentioned.

Reagent( $\mu\text{L}$ )	Test tube (T)	Control tube (C)
Supernatant	50 $\mu\text{L}$	50 $\mu\text{L}$
Reagent I	30 $\mu\text{L}$	30 $\mu\text{L}$
Reagent III	100 $\mu\text{L}$	100 $\mu\text{L}$
Mix thoroughly, keep at room temperature for 2 minutes, then determine the absorbance at 412 nm, record as $A_T$ and $A_C$ , calculate $\Delta A = A_T - A_C$		

### III. Calculation

#### 1. Tissues

##### 1) Protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 nmol TNB in the reaction system per minute every mg protein.

$$\text{AChE Enzyme activity (U/mg prot)} = [\Delta A \div \epsilon \div d \times V_C \times 10^9] \div (C_{pr} \times V_S \times V_{SU} \div V_{EN}) \div T$$

$$= 2255 \times \Delta A \div C_{pr}$$

##### 2) Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 nmol TNB in the reaction system per minute every g sample.

$$\text{AChE Enzyme activity (U/g fresh weight)} = [\Delta A \div \epsilon \div d \times V_C \times 10^9] \div (W \times V_S \div V_{TS} \times V_{SU} \div V_{EN}) \div T$$

$$= 2255 \times \Delta A \div W$$

#### 2. Bacteria and cells:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 nmol TNB in the reaction system per minute every  $10^4$  cells.

$$\text{AChE Enzyme activity (U/} 10^4 \text{ cell)} = [\Delta A \div \epsilon \div d \times V_C \times 10^9] \div (N \times V_S \div V_{TS} \times V_{SU} \div V_{EN}) \div T$$

$$= 2255 \times \Delta A \div N$$

#### 3. Serum:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 nmol TNB in the reaction system per minute every mL serum.

$$\text{AChE Enzyme activity (U/mL)} = [\Delta A \div \epsilon \div d \times V_C \times 10^9] \div (V_S \times V_{SU} \div V_{EN}) \div T$$

$$= 2255 \times \Delta A$$

$\epsilon$  : molar extinction coefficient of TNB,  $13.6 \times 10^4 \text{ L/mol/cm}$ ;

$V_C$  : Total volume of color reaction system (L),  $1\text{mL} = 0.001 \text{ L}$ ;

$10^9$  :  $1 \text{ mol} = 1 \times 10^9 \text{ nmol}$ ;

$V_{EN}$  : Total volume of enzymatic reaction,  $0.23\text{mL}$ ;

$V_{SU}$  : Supernatant volume,  $0.05\text{mL}$ ;

$V_{TS}$  : Extraction volume,  $1\text{mL}$ ;

$C_{pr}$  : Protein concentration,  $\text{mg/mL}$ ;

$W$  : Sample weight,  $\text{g}$ ;

$V_S$  : Sample volume,  $0.03\text{mL}$ ;

$T$  : Reaction time, 5 minutes;

$N$  : The number of cells extracted,  $10^4$ .

### Note

- During the determination process, the sample and the working fluid should be placed on ice to avoid denaturation and inactivation.
- When the absorbance  $\geq 1$ , it is recommended to dilute the sample.