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

BC8801-01 (50Tests/24Samples)

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

## Product Description

Acetylcholinesterase (AChE) is a serine hydrolytic enzyme, which is widely found in various animal tissues and serum. AChE catalyzes the hydrolysis of ACh, which plays an important role in the regulation of nerve conduction. AChE catalyzes ACh hydrolysis to generate choline, and choline can react with 2-nitrobenzoic acid (DTNB) to form 5-mercapto nitrobenzoic acid (TNB). TNB has an absorption peak at 412 nm, and AChE activity was calculated by measuring the absorbance increasing rate at 412 nm.

## Kit components

Reagent	Volume	Storage
Extraction solution	30mL	2-8°C
Reagent I	50mL	2-8°C
Reagent II	Powder x 2	2-8°C
Reagent III	6mL	2-8°C
Reagent IV	6mL	2-8°C

## Reagent Preparation

- 1. Reagent II:** Before use, add 2.6mL Reagent I to one Reagent II and fully dissolve it. The reagents can be dissolved at 2-8°C for one week.

## Reagents and Equipment Required but Not Provided

Spectrophotometer, low temperature centrifuge, water bath, adjustable pipette, 1 mL glass cuvette, mortar/homogenizer/cell ultrasonic crusher and distilled water.

## Operation Procedures

### I. Sample Preparation

- 1. Tissues:** According to the tissues mass (g): Extract solution volume (mL) is the ratio of 1:5-10 (suggest that take 0.1 g tissues and add 1 mL Extract solution) on the ice bath to homogenate. Centrifuge at 8000 g for 10 minutes at 4°C, take the supernatant for test.
- 2. Bacteria/cells:** According to the number of cells ( $10^4$ ), the proportion of Extract solution volume (mL) is 500-1000:1 (Suggest that add 1 mL of Extract solution to 5 million cells). Ultrasonic breaking (power 300W, ultrasonic 3s, interval 7s, total time 3minutes) on ice. Centrifuge at 8000 g for 10 minutes at 4°C, take the supernatant on ice for test.
- 3. Serum and other liquids:** Direct determination.

### II. Determination procedure:

1. Preheat the spectrophotometer for 30 min, adjust the wavelength to 412 nm, and set the counter to zero with distilled water.

## 2. Operation table

Reagent name (μL)	Test tube (A <sub>T</sub> )	Control tube (A <sub>C</sub> )
Sample	30	30
Reagent II	100	-
Accurate reaction in water at 37°C for 5 minutes.		
Reagent IV	100	100
Reagent II	-	100
Mix thoroughly, centrifuge at 1200 rpm for 5 minutes at room temperature. Pipet 50 μL of the supernatant into the new EP tube and add it separately.		
Reagent I	850	850
Reagent III	100	100
Mix thoroughly, stay for 2 minutes, then determine the absorbance at 412 nm, record as A <sub>T</sub> and A <sub>C</sub> , calculate $\Delta A = A_T - A_C$ . A control tube is required for each test tube.		

## III. Calculation

### 1. Tissues

#### a) 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.

$$\text{AChE activity (U/mg prot)} = \Delta A \div (\epsilon \times d) \times V_C \times 10^9 \div (C_{pr} \times V_s \times V_{SU} \div V_{EN}) \div T = 2255 \times \Delta A \div C_{pr}$$

#### b) 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.

$$\text{AChE activity (U/g mass)} = \Delta A \div (\epsilon \times d) \times V_C \times 10^9 \div (W \times V_s \div V_{TS} \times V_{SU} \div V_{EN}) \div T = 2255 \times \Delta A \div W$$

### 2. Bacterial 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<sup>4</sup> cells.

$$\text{AChE activity (U/10}^4 \text{ cell)} = \Delta A \div (\epsilon \times d) \times V_C \times 10^9 \div (N \times V_s \div V_{TS} \times V_{SU} \div V_{EN}) \div T = 2255 \times \Delta A \div N$$

### 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.

$$\text{AChE activity (U/mL)} = [\Delta A \div (\epsilon \times d) \times V_C \times 10^9] \div (V_S \times V_{SU} \div V_{EN}) \div T = 2255 \times \Delta A$$

$\epsilon$ : The molar extinction coefficient of TNB is  $13.6 \times 10^3$  L/mol/cm

d Light path of cuvette, 1 cm

Vc: Total volume of color reaction system (L), 1 mL=0.001 L

$10^9$ : Unit conversion factor. 1 mol= $1 \times 10^9$  nmol

V<sub>EN</sub>: Total volume of enzymatic reaction, 0.23 mL

V<sub>su</sub>: Supernatant volume, 0.05 mL

V<sub>TS</sub>: Extraction volume, 1 mL

C<sub>pr</sub>: Protein concentration, mg/mL

W: Sample weight, g

V<sub>s</sub>: Sample volume, 0.03 mL

T. Reaction time, 5 minute

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

**Note:**

1. During the determination process, the sample and the working fluid should be placed on ice to avoid denaturation and inactivation.
2. When the absorbance is more than 1, it is recommended to dilute the sample for determination.

For further details, contact us at