



Ver.240707

Acetylcholinesterase (AChE) Activity Assay Kit

BC8801-01 (50 Tests/24 Samples)

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 Acetylcholine (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. AChE activity is 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 × 2	2-8°C
Add 2.6mL Reagent I and dissolve completely. Unused reagent can be 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, 1 mL Glass Cuvette, adjustable pipette, Mortar/ Homogenizer and Distilled Water/Cell Ultrasonic Crusher.

Enzyme Extraction

1. Tissue: 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, 4°C for 10 minutes, take the supernatant for test.

Note: The supernatant should be placed on ice.

2. Bacteria/ Cells: According to the number of cells (10^4), the proportion of Extract solution volume (mL) is 500~1000=1:1 (Suggest that add 1 mL of Extract solution to 5 million cells). Ultrasonic breaking (power 300W, ultrasonic 3seconds, interval 7seconds, total time 3minutes) on ice; Then Centrifuge at 8000× g, 4°C for 10 minutes, take the supernatant on ice for test.

Note: The supernatant should be placed on ice.

3. Serum and other liquids: Direct determination

Determination Procedures

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	Test (A _T)	Control (A _C)
Sample	30μL	30μL
Reagent II	100μL	-
Incubate in a 37°C water bath for 5 minutes		
Reagent IV	100μL	100μL
Reagent II	-	100μL
Mix thoroughly and centrifuge at 12000 rpm for 5 minutes. Pipette 50μL supernatant into a new EP tube and add it separately.		
Reagent I	850μL	850μL
Reagent III	100μL	100μL
Mix thoroughly, keep at room temperature for 2 minutes. Determine absorption at 412nm, record as A _T and A _C . Calculate $\Delta A = A_T - A_C$		

Calculations

1. Tissue:

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.

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

$$= 2255 \times \Delta A \div \text{Cpr}$$

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.

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

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

2. Bacteria/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 10⁴ cells.

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

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

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.

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

$$= 2255 \times \Delta A$$

ϵ : The molar extinction coefficient of TNB is 13.6×10^3 L/mol/cm
 V_C : Total volume of color reaction system (L), 1 mL=0.001 L;
 10^{-9} : 1 mol= 1×10^9 nmol;
 V_{EN} : Total volume of enzymatic reaction, 0.23 mL;
 V_{SU} : Supernatant volume, 0.05 mL;
 V_{TS} : Extraction volume, 1 mL;
 C_{pr} : Protein concentration, mg/mL;
 W : Sample weight, g;
 V_S : Sample volume, 0.03 mL;
 T : Reaction time, 5 minutes;
 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 over than 1, it is recommended to dilute the sample for determination.