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Ver.260301

Acetylcholinesterase (AChE) Activity Assay Kit

BC8801-02 (100 Tests/48 Samples)

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

Product Description

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
Extract solution	60 mL	2-8°C
Reagent I	30 mL	2-8°C
Reagent II	Powder	2-8°C
Reagent III	3 mL	2-8°C
Reagent IV	Powder	2-8°C

Reagent Preparation

Reagent II

Add 6 mL distilled and gently mix to dissolve the contents. Unused reagents can be stored in aliquots at 4°C for 4 months.

Reagent IV

Add 6mL Reagent I to the powder and gently mix to dissolve the contents. The solution is stable for 2 weeks at 2–8°C and for 2 months at –20°C. Prepare aliquots before freezing to avoid multiple freeze-thaw cycles.

Reagents and Equipment Required but Not Provided

Spectrophotometer/microplate reader, Centrifuge, Water Bath, adjustable pipette, microcentrifuge tubes (EP tubes), micro glass cuvette/96, well flat-bottom plate, homogenizer/mortar and Distilled Water.

Protocol

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, 4°C for 10 minutes, take the supernatant for test.
2. **Bacteria or cells:** According to the number of cells (10⁴), 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 3 seconds, interval 7 seconds, total time 3 minutes) on ice; Then Centrifuge at 8000 g, 4°C for 10 minutes, take the supernatant on ice for test.
3. **Serum and other liquids:** Direct determination.

II. Determination procedure

1. Preheat the spectrophotometer/microplate reader 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 (A_T)	Control tube (A_C)
Sample	15	15
Reagent II	50	-
Incubate at 37°C for 5 minutes		
Reagent IV	50	50
Reagent II	-	50
Mix thoroughly, centrifuge at 12000 rpm for 5 minutes at room temperature. Pipette 10 μL of the supernatant into the new EP tube/96 well flat bottom and add it separately		
Reagent I	170	170
Reagent III	20	20
Mix thoroughly, stay for 2 minutes, determine the absorbance at 412 nm, record as A_T and A_C , Calculate $\Delta A = A_T - A_C$.		

Calculations

A. Micro glass cuvette

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.

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

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

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.

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

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

2. Bacteria 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.

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

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

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 $13.6 \times 10^3 \text{ L/mol/cm}$

d : Light path of cuvette, 1 cm

V_C : Total volume of color reaction system (L), $0.2 \text{ mL} = 2 \times 10^{-4} \text{ L}$

10^9 : Unit conversion factor, $1 \text{ mol} = 1 \times 10^9 \text{ nmol}$;

V_{EN} : Total volume of enzymatic reaction, 0.115 mL

V_{SU} : Supernatant volume, 0.01 mL;

V_{TS} : Extraction volume, 1 mL;

Cpr : Protein concentration, mg/mL;

W : Sample weight, g;

V_S : Sample volume, 0.015 mL

T : Reaction time, 5 minutes;

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

B. 96 well flat-bottom plate:

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.

$$\text{AChE Enzyme activity (U/mg prot)} = [\Delta A \div (\epsilon \times d) \times V_C \times 10^9] \div (C_{pr} \times V_S \times V_{SU} \div V_{EN}) \div T \\ = 3758 \times \Delta A \div C_{pr}$$

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.

$$\text{AChE Enzyme activity (U/g fresh weight)} = [\Delta A \div (\epsilon \times d) \times V_C \times 10^9] \div (W \times V_S \div V_{TS} \times V_{SU} \div V_{EN}) \div T \\ = 3758 \times \Delta A \div W$$

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 10⁴ cells.

$$\text{AChE Enzyme activity (U/10}^4 \text{ cell)} = [\Delta A \div (\epsilon \times d) \times V_C \times 10^9] \div (N \times V_S \div V_{TS} \times V_{SU} \div V_{EN}) \div T \\ = 3758 \times \Delta A \div N$$

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.

$$\text{AChE Enzyme activity (U/mL)} = [\Delta A \div (\epsilon \times d) \times V_C \times 10^9] \div (V_S \times V_{SU} \div V_{EN}) \div T \\ = 3758 \times \Delta A$$

ϵ : The molar extinction coefficient of TNB is 13.6×10³ L/mol/cm

d : Light path of cuvette, 0.6 cm

V_C : Total volume of color reaction system (L), 0.2 mL=2× 10⁻⁴L

10⁹ : Unit conversion factor, 1 mol=1×10⁹nmol;

V_{EN} : Total volume of enzymatic reaction, 0.115 mL

V_{SU} : Supernatant volume, 0.01 mL;

V_{TS} : Extraction volume, 1 mL;

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

W : Sample weight, g;

V_S : Sample volume, 0.015 mL

T : Reaction time, 5 minutes;

N : The number of cells extracted, 10⁴.

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.
3. Each sample requires a Test tube and a Control tube.