Acetylcholinesterase (AchE) Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: BC2025

Size: 100T/48S

Components:

Extract Solution: Liquid 60 mL ×1. Storage at 2-8℃.

Reagent I: Liquid 30 mL×1. Storage at 2-8℃.

Reagent II: Powder ×2. Storage at 2-8℃. Add 2.6 mL reagent I before use and dissolve fully by shock, the unused reagent could be stored at 2-8℃ for 1 week.

Reagent III: Liquid 3 mL×1. Storage at 2-8℃.

Reagent IV: Liquid 6 mL×1. Storage at 2-8℃.

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.

Reagents and Equipment Required but Not Provided.

Refrigerated Centrifuge, Water-Bath, Transferpettor, Spectrophotometer/Microplate Reader, Micro Glass Cuvette/96 Well Flat-Bottom Plate, Homogenizer/Mortar and Distilled Water.

Procedure

I. Enzyme extraction:

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 8000g, 4℃ for 10minutes, take the supernatant for test.

2. Bacteria and 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 m illion cells). Ultrasonic breaking (power 300w, ultrasonic 3s, interval 7s, total time 3minutes) on ice; Then Centrifuge at 8000g, 4℃ for 10minutes, 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 the spectrophotometer needs to be zeroed with distilled water.

2. Operation table:

Reagent name (μL)	Test tube (A_T)	Control Tube (A_C)
Sample	15	15
Reagent II	50	-
Accurate reaction in water bath at 37°C for 5 minutes.		
Reagent IV	50	50
Reagent II	-	50
Mix thoroughly, centrifuge at 12000 rpm for 5 minutes. Pipet 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, then use micro glass cuvette/96 well flat-bottom plate to determine the absorbance at 412 nm, record as A_T and A_C, calculate $\Delta A=A_T-A_C$.		

III. Calculation formula of AchE activity

A. Micro glass cuvette:

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.

AchE Enzyme activity (U/mg prot)=$[\Delta A\div\varepsilon\div d\times V_C\times10^9]\div(Cpr\times V_S\times V_{SU}\div V_{EN})\div T$

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

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.

AchE Enzyme activity (U/g fresh weight)=$[\Delta A\div\varepsilon\div d\times V_C\times10^9]\div(W\times V_S\div V_{TS}\times V_{SU}\div V_{EN})\div T$

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

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.

AchE Enzyme activity (U/10^4 cell)=$[\Delta A\div\varepsilon\div d\times V_C\times10^9]\div(N\times V_S\div V_{TS}\times V_{SU}\div V_{EN})\div T$

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

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.

AchE Enzyme activity(U/mL)=$[\Delta A\div\varepsilon\div d\times V_C\times10^9]\div(V_S\times V_{SU}\div V_{EN})\div T$

$=2255\times\Delta A$

ε: The molar extinction coefficient of TNB is 13.6×10^3 L/mol/cm;

V_C: Total volume of color rendering reaction system (L), 1 mL=2×10^{-4} L;

10^9:1 mol=1×10^9 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:

Change d-1cm in the above formula to d-0.6cm for calculation.

Note:

1. During the measurement 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 determi nation.

Recent Product Citations:

[1] Wensu Han,Yemeng Yang,Jinglin Gao,et al. Chronic toxicity and biochemical response of Apis cerana cerana (Hymenoptera: Apidae) exposed to acetamiprid and propiconazole alone or combined. Ecotoxicology.May 2019; 28(4):399-411.(IF2.46)

[2] Hao Song,Liping Huang,Yuping Li,et al. Neuroprotective effects of cordycepin inhibit Aβ-induced apoptosis in hippocampal neurons. NeuroToxicology. September 2018;(IF3.263)

[3] Xiao Hui Xu,Yinghui Guo,Hongwei Sun,et al.Effects of Phytase Transgenic Maize on the Physiological and Biochemical Responses and the Gut Microflora Functional Diversity of Ostrinia furnacalis. Scientific Reports. March 2018;(IF4.011)

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BC2130/BC2135 Acid Phosphatase(ACP) Activity Assay Kit

BC2140/BC2145 Alkaline Phosphatase(AKP/ALP) Activity Assay Kit

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