Acetylcholinesterase (AchE) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: NA0725

Size: 50T/24S

Components:

Extract solution: Liquid 25 mL ×1. Storage at 4℃.

Reagent I: Liquid 55 mL×1. Storage at 4℃.

Reagent II: Powder×1. Storage at 4℃. Add 5.2 mL reagent I before use and dissolve fully by oscillation.

Reagent III: Powder×1. Storage at 4℃. Add 5.2 mL reagent I before use and dissolve fully by oscillation.

Reagent IV: Powder×1. Storage at 4℃. Add 5 mL distilled water to dissolve before use.

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, spectrophotometer, 1 mL glass cuvette, transferpettor, mortar/ homogenizer 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. C entrifuge at 8000 g, 4℃ for 10 minutes, take the supernatant for test.

2. Bacteria and cells: According to the number of cells (10^4), the proportion of Extract solution v olume (mL) is 500~1000=1:1 (Suggest that add 1 mL of Extract solution to 5 million cells). Ultr asonic breaking (power 300W, ultrasonic 3s, interval 7s, total time 3 min) on ice; Then Centrifug e at 8000 g, 4℃ for 10 minutes, take the supernatant on ice for test.

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 cou nter to zero with distilled water.

2. Operation table:

Reagent name (μL)	Test tube (A_T)	Control Tube (A_C)
Sample	30	30
Reagent II	100	-
Accurate reaction in water bath at 37℃ for 5 minutes.		
Reagent IV	100	100
Reagent II	-	100
Mix thoroughly, centrifuge at 12000 rpm for 5 minutes. Pipet 50 μL of the supernatant into the new EP tube and add it separately.		
Supernatant	50	50
Reagent I	850	850
Reagent III	100	100
Mix thoroughly, stay for 2 minutes, then determine the absorbance at 412 nm, recoard 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 ge neration 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 ge neration 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 ge neration 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 ge neration 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 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;
Cpr: 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 determinaton 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.

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)

Related products:

NA0806/NA0564 Carboxylesterase (CarE) Activity Assay Kit
NA0719/NA0477 Acid Phosphatase (ACP) Activity Assay Kit
NA0718/NA0498 Alkaline Phosphatase (AKP/ALP) Activity Assay Kit