PRODUCT INFORMATION & MANUAL

Acetylcholinesterase/ ACHE Activity Assay Kit (Colorimetric)

NBP3-24453

For research use only.
Not for diagnostic or therapeutic procedures.

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Acetylcholinesterase/ACHE Activity Assay Kit (Colorimetric)

Catalog No: NBP3-24453

Method: Colorimetric method

Specification: 96T (Can detect 96 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 1.225 U/mL

Detection range: 1.225-490 U/mL

Average intra-assay CV (%): 4.7

Average inter-assay CV (%): 9.3

Average recovery rate (%): 104

- This kit is for research use only.
- Instructions should be followed strictly, changes of operation may result in unreliable results.
- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to measure acetylcholinesterase (AchE) content in serum, plasma, animal tissue samples.

▲Background

Acetylcholinesterase (AchE) (EC 3.1.1.7) is a serine hydrolase with monomer, dimer and tetramer structures, also known as G1, G2 and G4, containing one, two or four catalytic subunits, mainly exist in neuromuscular junctions and cholinergic synapses in the brain. The main biological role of AchE is the rapid hydrolysis of the neurotransmitter acetylcholine into acetic acid and choline to terminate the impulsive transmission of cholinergic synapses.

▲Detection principle

AchE catalyzes the hydrolysis of acetylcholine to form choline, and choline react with dithio p-nitrobenzoic acid (DTNB) to form 5-mercapto-nitrobenzoic acid (TNB). TNB has an absorption peak at 412nm. And the activity of AchE is calculated by measuring the increasing rate of absorbance at 412nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Lysis Buffer	50 mL × 2 vials	2-8℃ , 12 months
Reagent 2	Buffer Solution	30 mL × 1 vial	2-8℃ , 12 months
Reagent 3	Chromogenic Agent	Powder × 1 vial	2-8℃ , 12 months, shading light
Reagent 4	Substrate	Powder × 1 vial	2-8℃ , 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Microplate reader (412 nm), Micropipettor, Incubator, Vortex mixer, Centrifuge

Reagents:

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key point of the assay

The samples could not contain chelating agents such as EGTA and EDTA, or reductive substances such as DTT and mercapto ethanol.

Pre-assay preparation

▲Reagent preparation

1. Preparation of reagent 3 working solution

Dissolve a vial of reagent 3 powder with 22 mL of reagent 2 fully before use. The prepared solution can be stored for 7 days with shading light.

2. Preparation of reagent 4 working solution

Dissolve a vial of reagent 4 powder with 1.3 mL of reagent 2 fully before use. The prepared solution can be stored for 7 days with shading light.

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

Sample requirements

The samples could not contain chelating agents such as EGTA and EDTA, or reductive substances such as DTT and mercapto ethanol.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (1.225-490 U/mL). When the OD value (A2) at 330 seconds is more than 2.5 in pre-experiment, the sample should be diluted.

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Mouse serum	8-20
Mouse plasma	4-10
Human serum	4-10
Human plasma	4-10
Rat serum	4-10
Dog serum	4-10
Horse serum	2-8
10% Mouse liver tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse brain tissue homogenate	2-8
10% Crucian carp muscle tissue homogenate	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92
E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	S93
F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86	S94
G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87	S95
H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88	S96

[Note]: S1-S96, sample wells.

▲Detailed operating steps

1. Take 20 μL of sample to the well of microplate.
2. Add 170 μL of reagent 3 working solution to each well.
3. Add 10 μL of reagent 4 working solution to each well.
4. Mix fully for 5 s with microplate reader, measure the changes in absorbance at 412 nm within 5 min. The OD value of 30 seconds and 330 seconds were ·recorded as A_1 and A_2, respectively. $\Delta A=A_2-A_1$.

▲Summary operation table

	Sample well
Sample (μL)	20
Reagent 3 working solution (μL)	170
Reagent 4 working solution (μL)	10
Mix fully, measure the changes in absorbance at 412 nm within 5 min. The OD value of 30 seconds and 330 seconds were recorded as A_1 and A_2, respectively.	

▲Calculation

1. Tissue sample

1) Calculate according to the protein concentration of sample

Definition: The enzymatic amount that catalyzes the production of 1 nmol TNB by 1 mg of protein per minute is defined as 1 unit.

$$\text{AchE activity (U/mg prot)} = (\Delta A \times \frac{V_{total}}{\varepsilon \times d} \times 10^9) \div (C_{pr} \times V_{sample}) \div T \times f$$

$$= 245 \times \Delta A \div C_{pr} \times f$$

2) Calculate according to the weight of sample

Definition: The enzymatic amount that catalyzes the production of 1 nmol TNB by 1 g of sample per minute is defined as 1 unit.

$$\text{AchE activity (U/mg fresh weight)} = (\Delta A \times \frac{V_{total}}{\varepsilon \times d} \times 10^9) \div \frac{W \times V_{sample}}{V_{total\ sample}} \div T \times f$$

$$= 245 \times \Delta A \div W \times f$$

2. Serum (plasma) samples:

Definition: The enzymatic amount that catalyzes the production of 1 nmol TNB by 1 mL of serum (plasma) per minute is defined as 1 unit.

$$\text{AchE activity (U/mL)} = (\Delta A \times \frac{V_{total}}{\varepsilon \times d} \times 10^9) \div V_{sample} \div T \times f$$

$$= 245 \times \Delta A \times f$$

Note:

ε: molar extinction coefficient of TNB, 13.6×10^{4} L /mol/cm;

d: optical path of the 96 wells microplate, 0.6 cm;

V total: total volume of reaction system, 2×10^{-4} L;

V sample: volume of sample added into the reaction system, 20 μL=2×10^{-2} mL;

V total sample: volume of the added extraction solution, 1 mL;

10^{9}: unit conversion, 1 mol = 10^{9} nmol;

T: reaction time, 5 min;

W: weight of sample, g;

C_{pr}: concentration of protein in sample, mg/mL;

f: dilution factor of sample before test.

Appendix I Data

▲Example analysis

Dilute rat serum with PBS (0.01 M, pH 7.4) for 5 times, take 20 μL of diluted sample and carry the assay according to the operation table. The results are as follows:

The average OD value at 30 s and 330 s are A1 (0.297) and A2 (0.405), ΔA= A2-A1=0.108, and the calculation result is:

$$\text{AchE activity (U/mL)} = 245 \times 0.108 \times 5 = 132.3 \text{ U/mL}$$

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80℃ for a month.

▲ Plasma

Take fresh blood into the tube which has anticoagulant (Heparin is used as anticoagulant), centrifuge at 700-1000 g for 10 min at 4℃ . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80℃ for a month.

▲ Tissue sample

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8℃ . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 8000 g at 4℃ . Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for a month.

Note:

1. Homogenized medium: Reagent 1.

2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

Appendix III References

1. Colovic M B, Krstic D Z, Lazarevic-Pasti T D, et al. Acetylcholinesterase inhibitors: pharmacology and toxicology[J]. Curr Neuropharmacol, 2013, 11(3): 315-335.
2. Lane R M, Kivipelto M, Greig N H. Acetylcholinesterase and Its Inhibition in Alzheimer Disease[J]. Clinical Neuropharmacology, 2004, 27(3): 141-149.