（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K174-M

Specification：48T(48 samples)/96T(96 samples)

Measuring instrument：Microplate reader（412 nm）

Detection range：1.225-490 U/mL

Elabscience® Acetylcholinesterase (AchE) Activity Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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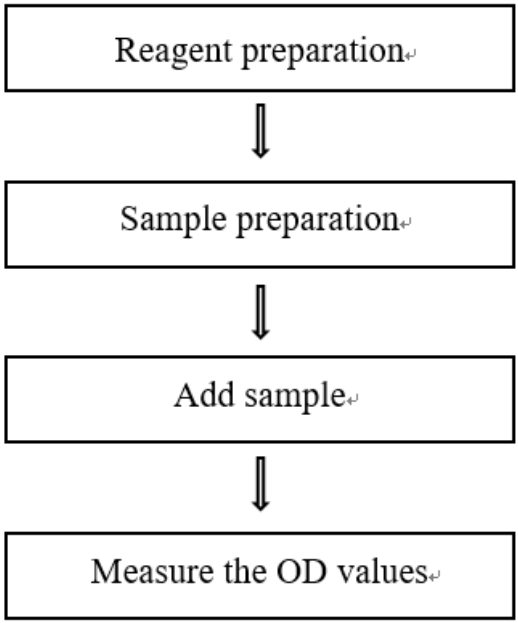
Assay summary



Intended use

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

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 412 nm. And the activity of AchE is calculated by measuring the increasing rate of absorbance at 412 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Lysis Buffer	50 mL ×1 vial	50 mL ×2 vials	2-8 ℃, 12 months
Reagent 2	Buffer Solution	15 mL ×1 vial	30 mL ×1 vial	2-8 ℃, 12 months
Reagent 3	Chromogenic Agent	Powder ×1 vial	Powder ×1 vial	2-8 ℃, 12 months, shading light
Reagent 4	Substrate	Powder ×1 vial	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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

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

Reagent preparation

① Incubate buffer solution at 37 ℃ for 30 min. Equilibrate other reagents to room temperature before use.

② The preparation of chromogenic working solution：

48T: Dissolve one vial of chromogenic agent with 11 mL of buffer solution, mix well. Store at 2-8 ℃ for 7 days protected from light.

96T: Dissolve one vial of chromogenic agent with 22 mL of buffer solution, mix well. Store at 2-8 ℃ for 7 days protected from light.

③ The preparation of substrate working solution：

48T: Dissolve one vial of substrate with 0.7 mL of buffer solution, mix well. Store at 2-8 ℃ for 7 days protected from light.

96T: Dissolve one vial of substrate with 1.3 mL of buffer solution, mix well. Store at 2-8 ℃ for 7 days protected from light.

Sample preparation

① Sample preparation:

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Tissue samples:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL lysis buffer with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cells:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 200 μL lysis buffer with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

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

Operating steps

① Take 20 μL of sample to the well of microplate.

② Add 170 μL of chromogenic working solution to each well.

③ Add 10 μL of substrate 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. $\triangle A=A_2-A_1$.

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.

$$\text{AchE activity (U/mgprot)} = (\triangle A \times \frac{V_{total}}{\varepsilon \times d} \times 10^9) \div (C_{pr} \times V_{sample}) \div T \times f = 245 \times \triangle A \div C_{pr} \times f$$

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.

$$\text{AchE activity (U/g fresh weight)} = (\triangle A \times \frac{V_{total}}{\varepsilon \times d} \times 10^9) \div \frac{W \times V_{sample}}{V_{total\ sample}} \div T \times f = 245 \times \triangle A \div W \times f$$

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.

$$\text{AchE activity (U/mL)} = (\triangle A \times \frac{V_{total}}{\varepsilon \times d} \times 10^9) \div V_{sample} \div T \times f = 245 \times \triangle A \times f$$

3. Cell 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.

$$\text{AchE activity (U/mgprot)} = (\triangle A \times \frac{V_{total}}{\varepsilon \times d} \times 10^9) \div (C_{pr} \times V_{sample}) \div T \times f = 245 \times \triangle A \div C_{pr} \times f$$

[Note]

ε: molar extinction coefficient of TNB, 1.36×10^4 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 Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	10.20	88.50	264.00
%CV	5.2	4.6	4.3

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mL)	10.20	88.50	264.00
%CV	9.1	9.3	9.5

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 104%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/mL)	138.5	252	381.3
Observed Conc. (U/mL)	146.8	257.0	396.6
Recovery rate (%)	106	102	104

Sensitivity

The analytical sensitivity of the assay is 1.225 U/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Appendix II Example Analysis

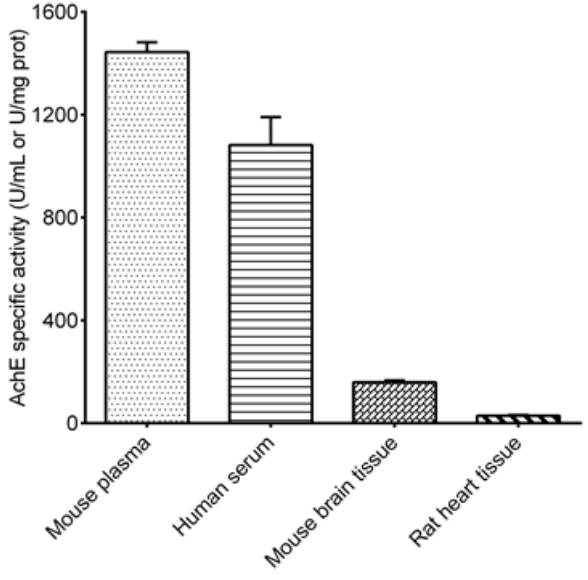
Example analysis:

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 steps. The results are as follows:

The average OD value at 30 s and 330 s are A_1 (0.297) and A_2 (0.405), $\triangle A = A_2 - A_1 = 0.108$, and the calculation result is:

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

Detect mouse plasma (dilute for 5 times), human serum (dilute for 10 times), 10% mouse brain tissue homogenate (the concentration of protein is 6.879 mgprot/mL, dilute for 3 times) and 10% rat heart tissue homogenate (the concentration of protein is 3.410 mgprot/mL) according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.