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

Catalog No：E-BC-K052-M

Specification：48T(44 samples)/96T(92 samples)

Measuring instrument：Microplate reader（505-535 nm）

Detection range：2.17-71.33 U/mL

Elabscience®Cholinesterase (CHE) 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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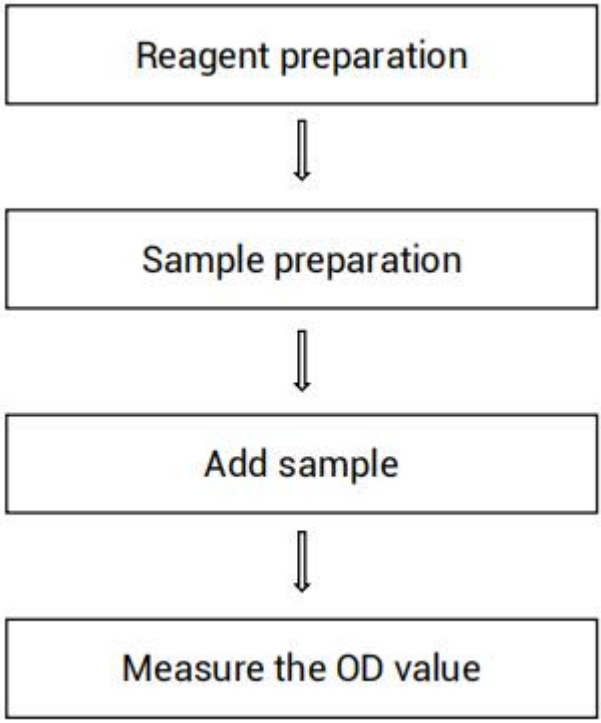
Assay summary



Intended use

This kit can be used for detection of cholinesterase (CHE) activity in serum (plasma) and animal tissue samples.

Detection principle

Cholinesterase breaks down acetylcholine into choline and acetic acid. Acetylcholine that is not hydrolyzed by cholinesterase reacts with hydroxylamine to form acetamidamine. It reacts in an acidic solution to form a brown-red hydroxamate iron complex. The color depth is directly proportional to the amount of remaining acetylcholine, which can be colorimetrically quantified. Cholinesterase activity was calculated.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	15 mL × 1 vial	30 mL × 1 vial	2-8°C, 12 months
Reagent 2	Substrate	Powder × 1 vial	Powder × 2 vials	2-8°C, 12 months, shading light
Reagent 3	Diluent A	1.5 mL ×1 vial	1.5mL × 2 vials	2-8°C, 12 months
Reagent 4	Chromogenic Agent A	Powder × 1 vial	Powder × 1 vial	2-8°C, 12 months
Reagent 5	Alkaline Reagent	10 mL ×1 vial	20 mL × 1 vial	2-8°C, 12 months
Reagent 6	Acid Reagent	12 mL × 1 vial	24 mL × 1 vial	2-8°C, 12 months
Reagent 7	Protein Precipitator	10 mL × 1 vial	20 mL × 1 vial	2-8°C, 12 months
Reagent 8	Chromogenic Agent B	Powder × 1 vial	Powder × 1 vial	2-8°C, 12 months, shading light
Reagent 9	Diluent B	0.5 mL × 1 vial	1 mL × 1 vial	2-8°C, 12 months

	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

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 (505-535 nm, optimum wavelength: 520 nm), Centrifuge, Vortex mixer, 37°C water bath

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

Size 1(48 T)：

① Equilibrate all reagents to room temperature before use.

② The preparation of 80 μmol/mL substrate stock solution：
Dissolve one vial of substrate powder with 1 mL of diluent A. Mix well to dissolve. Store at 2-8°C for 1 week protected from light.

③ The preparation of substrate application solution：
For each well，prepare 60 μL of substrate application solution (mix well 6 μL of substrate stock solution and 54 μL of buffer solution). The substrate application solution should be prepared on spot and used up in the same day.

④ The preparation of chromogenic agent A stock solution：

Dissolve one vial of chromogenic agent A with 10 mL of double distilled water. Mix well to dissolve. Store at 2-8°C for 3 months.

⑤ The preparation of chromogenic agent A application solution：

For each well， prepare 200 μL of chromogenic agent A application solution (mix well 100 μL of chromogenic agent A stock solution and 100 μL of alkaline reagent). The substrate application solution should be prepared on spot and used up in the same day.

⑥ The preparation of diluent B application solution：

Dilute 250 μL of diluent B with 9.75 mL of double distilled water, mix well. Store at 2-8°C for 6 months.

⑦ The preparation of chromogenic agent B application solution：

Dissolve one vial of chromogenic agent B with 10 mL of diluent B application solution. Mix well to dissolve. Store at 2-8°C for 3 months protected from light.

Size 2(96 T)：

① Equilibrate all reagents to room temperature before use.

② The preparation of 80 μmol/mL substrate stock solution：

Dissolve one vial of substrate powder with 1 mL of diluent A. Mix well to dissolve. Store at 2-8°C for 1 week protected from light.

③ The preparation of substrate application solution：

For each well，prepare 60 μL of substrate application solution (mix well 6 μL of substrate stock solution and 54 μL of buffer solution). The substrate application solution should be prepared on spot and used up in the same day.

④ The preparation of chromogenic agent A stock solution：

Dissolve one vial of chromogenic agent A with 20 mL of double distilled water. Mix well to dissolve. Store at 2-8°C for 3 months.

⑤ The preparation of chromogenic agent A application solution：

For each well， prepare 200 μL of chromogenic agent A application solution (mix well 100 μL of chromogenic agent A stock solution and 100 μL of alkaline reagent). The substrate application solution should be prepared on spot and used up in the same day.

⑥ The preparation of diluent B application solution：
Dilute 500 μL of Diluent B with 19.5 mL of double distilled water, mix well. Store at 2-8°C for 6 months.

⑦ The preparation of chromogenic agent B application solution：
Dissolve one vial of chromogenic agent B with 20 mL of diluent B application solution. Mix well to dissolve. Store at 2-8°C for 3 months 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°C for a month.

Tissue sample:

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 normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 minutes at 4°C 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
Human serum	2-3
Human plasma	2-3
Mouse serum	2-3
Mouse plasma	2-3
10% Rat brain tissue homogenate	1
10% Rat spleen tissue homogenate	1
10% Rat heart tissue homogenate	1
10% Rat lung tissue homogenate	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① The brown-red iron complex after reaction is unstable, and the colorimetry must be completed within 20 minutes.

② There should be no bubbles in the wells of the microplate when measuring the OD value.

Operating steps

① Blank tube: Take 80 μL of double distilled water to the 2 mL EP tube.
Control tube: Take 20 μL of double distilled water and 60 μL of substrate application solution to the 2 mL EP tube.
Sample tube: Take 20 μL of sample and 60 μL of substrate application solution to the 2 mL EP tube.

② Add 150 μL of buffer solution to each tube.

③ Mix fully and incubate at 37°C for 20 min.

④ Successively add 200 μL of chromogenic agent A application solution, 150 μL of acid reagent, 100 μL of protein precipitator, 100 μL of chromogenic agent B application solution to each tube and mix fully.

⑤ Centrifuge at 2300 g for 10 min.

⑥ Take 250 μL of supernatant to the corresponding wells of microplate, measure the OD value of each well at 520 nm with microplate reader.

Calculation

The sample:

1. Serum (plasma) sample:

Unit definition: The amount of CHE in 1 mL of serum or plasma that react with substrate in 20 minute at 37°C and decompose 1 μmol acetylcholine is defined as 1 unit.

$$\text{CHE activity (U/mL)} = \frac{\Delta A_1 - \Delta A_2}{\Delta A_1} \times C \times \frac{V_1}{V_2} \times f$$

2. Tissue sample:

Unit definition: The amount of CHE in 1 mg of tissue protein that react with substrate in 20 minute at 37°C and decompose 1 μmol acetylcholine is defined as 1 unit.

$$\text{CHE activity (U/mgprot)} = \frac{\Delta A_1 - \Delta A_2}{\Delta A_1} \times C \times \frac{V_1}{V_2} \div C_{pr} \times f$$

[Note]

ΔA_1: $OD_{Control} - OD_{Blank}$.

ΔA_2: $OD_{Sample} - OD_{Blank}$.

C: The concentration of substrate application solution, 8 μmol/mL.

V_1: The volume of substrate application solution, 0.06 mL.

V_2: The volume of sample added to the reaction, 0.02 mL.

C_{pr}: Concentration of protein in sample, mgprot/mL.

f: Dilution factor of sample before tested.

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)	8.50	24.60	58.50
%CV	2.5	2.3	2.4

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)	8.50	24.60	58.50
%CV	5.9	6.8	6.5

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 102%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/mL)	16.4	33.6	60.5
Observed Conc. (U/mL)	16.2	34.6	62.9
recovery rate(%)	99	103	104

Sensitivity

The analytical sensitivity of the assay is 2.17 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 calcμLating the corresponding concentration.

Appendix Π Example Analysis

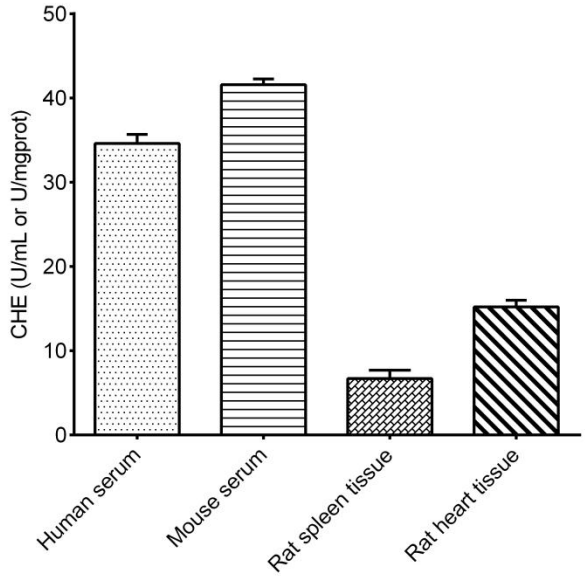
Example analysis：

For human plasma, dilute for 2 times, and carry the assay according to the operation steps. The results are as follows:

the average OD value of the blank is 0.044, the average OD value of the sample is 0.236, , the average OD value of the control is 0.426, and the calculation result is:

CHE activity (U/mL) = ((0.426 – 0.044) - (0.236 – 0.044)) ÷ (0.426 – 0.044) × 8 × 0.06 ÷ 0.02 × 2= 23.85 U/mL

Detect human serum (dilute for 2 times), mouse serum (dilute for 2 times), 10% rat spleen tissue homogenate (the concentration of protein is 8.60 mgprot/mL) and 10% rat heart tissue homogenate (the concentration of protein is 4.52 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 carefµLly 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 shoµLd 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 resµLts 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 calcµLate the possible usage of sample and reserve sufficient samples before use.