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

Catalog No：E-BC-K052-S

Specification：50 assays(48 samples)/100 assays(96 samples)

Measuring instrument：Spectrophotometer（520 nm）

Detection range：1.17-160 U/mL

Elabscience®Cholinesterase (CHE) Activity Assay Kit

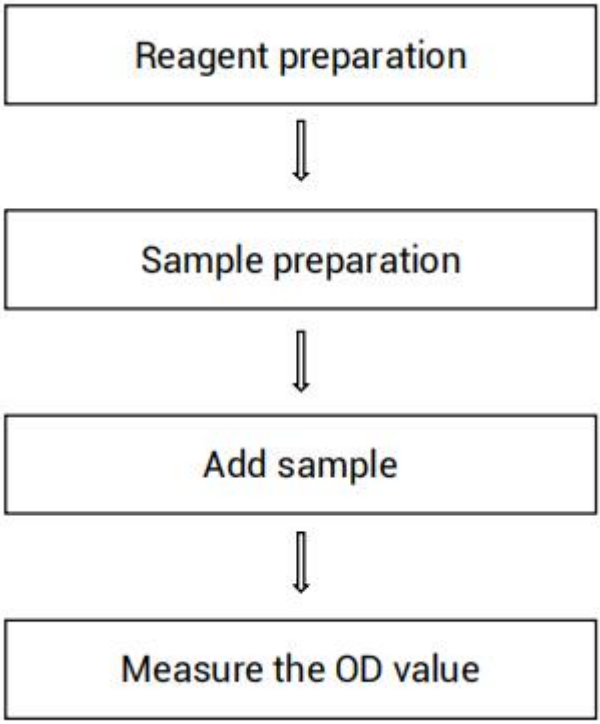
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

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.

Table of contents

Assay summary



Intended use

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

Detection principle

Cholinesterase breaks down acetylcholine into choline and acetic acid. Acetylcholine that is not hydrolyzed by cholinesterase reacts with basic 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 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Buffer Solution	60 mL × 1 vial	60 mL × 2 vials	2-8°C, 12 months
Reagent 2	Substrate	Powder × 1 vial	Powder × 2 vials	2-8°C, 12 months, shading light
Reagent 3	Diluent 1	5 mL ×1 vial	10 mL × 1 vial	2-8°C, 12 months
Reagent 4	Chromogenic Agent 1	Powder × 1 vial	Powder × 1 vial	2-8°C, 12 months
Reagent 5	Alkali Reagent	30 mL ×1 vial	60 mL × 1 vial	2-8°C, 12 months
Reagent 6	Acid Reagent	30 mL × 1 vial	60 mL × 1 vial	2-8°C, 12 months
Reagent 7	Protein Precipitator	20 mL × 1 vial	40 mL × 1 vial	2-8°C, 12 months
Reagent 8	Chromogenic Agent 2	Powder × 1 vial	Powder × 1 vial	2-8°C, 12 months, shading light
Reagent 9	Diluent 2	1 mL × 1 vial	2 mL × 1 vial	2-8°C, 12 months

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:

Spectrophotometer (520 nm), Micropipettor, Centrifuge, Incubator, Water bath, Vortex mixer

Reagents:

Double distilled water, Normal saline (0.9% NaCl)

Reagent preparation

Size 1(50 assays)：

① Equilibrate all reagents to room temperature before use.

② Preparation of 80 μmol/mL substrate stock solution：
Dissolve one vial of substrate powder with 5 mL of Diluent 1. Mix well to dissolve. Store at 2-8°C for 1 week protected from light.

③ Preparation of substrate application solution：
For each well， prepare 250 μL of substrate application solution (mix well 25 μL of substrate stock solution and 225 μL of buffer solution). The substrate application solution should be prepared on spot. Store at 2-8°C for 1day.

④ Preparation of chromogenic agent 1 stock solution：
Dissolve one vial of chromogenic agent 1 with 30 mL of double distilled water. Mix well to dissolve. Store at 2-8°C for 3 months.

⑤ Preparation of chromogenic agent 1 application solution：

For each well， prepare 1000 μL of chromogenic agent 1 application solution (mix well 500 μL of chromogenic agent 1 stock solution and 500 μL of alkali reagent). The substrate application solution should be prepared on spot. Store at 2-8°C for 1 day.

⑥ Preparation of diluent 2 application solution：
Dilute 0.75 mL of diluent 2 with 29.25 mL of double distilled water, mix well. Store at 2-8°C for 6 months.

⑦ Preparation of chromogenic agent 2 application solution：
Dissolve one vial of chromogenic agent 2 with 30 mL of diluent 2 application solution. Mix well to dissolve. Store at 2-8°C for 3 months protected from light.

Size 2(100 assays)：

① Equilibrate all reagents to room temperature before use.

② Preparation of 80 μmol/mL substrate stock solution：
Dissolve one vial of substrate powder with 5 mL of Diluent 1. Mix well to dissolve. Store at 2-8°C for 1 week protected from light.

③ Preparation of substrate application solution：
For each well，prepare 250 μL of substrate application solution (mix well 25 μL of substrate stock solution and 225 μL of buffer solution). The substrate application solution should be prepared on spot. Store at 2-8°C for 1 day.

④ Preparation of chromogenic agent 1 stock solution：
Dissolve one vial of chromogenic agent 1 with 60 mL of double distilled water. Mix well to dissolve. Store at 2-8°C for 3 months.

⑤ Preparation of chromogenic agent 1 application solution：
For each well，prepare 1000 μL of chromogenic agent 1 application solution (mix well 500 μL of chromogenic agent 1 stock solution and 500 μL of alkali reagent). The substrate application solution should be

prepared on spot. Store at 2-8°C for 1 day.

⑥ Preparation of diluent 2 application solution：
Dilute 1.5 mL of diluent 2 with 58.5 mL of double distilled water, mix well. Store at 2-8°C for 6 months.

⑦ Preparation of chromogenic agent 2 application solution：
Dissolve one vial of chromogenic agent 2 with 60 mL of diluent 2 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 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).

Cell (adherent or suspension) sample:

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 300-500 μL normal saline (0.9% NaCl)

with a ultrasonic cell disruptor at 4°C.

④ Centrifuge at 10000 × g for 10 minutes 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
SH-SY5Y cells	1
10% Mouse brain tissue homogenate	1
10% Rat brain tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Rat spleen tissue homogenate	1
10% Mouse liver 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 shoμLd be no bubbles in the wells of the microplate when measuring the OD value.

Operating steps

For serum (plasma), tissue and cells samples：

① Blank tube: Take 0.3 mL of double distilled water to the 5 mL tube.
Sample tube: Take 0.05 mL of sample and 0.25 mL of substrate application solution to the 5 mL tube.
Control tube: Take 0.05 mL of double distilled water and 0.25 mL of substrate application solution to the 5 mL tube.

② Add 0.5 mL of buffer solution to each tube and mix fully.

③ Incubate at 37°C for 20 min.

④ Successively add 1 mL of chromogenic agent 1 application solution, 0.5 mL of acid reagent, 0.25 mL of protein precipitator, 0.5 mL of chromogenic agent 2 application solution and mix fully.

⑤ Centrifuge at 2325×g for 10 min, then take the supernatant.

⑥ Set the spectrophotometer to zero with blank tube and measure the OD values of each tube at 520 nm with 1 cm optical path cuvette.

For whole blood samples：

① Blank tube: Take 0.35 mL of double distilled water to the 5 mL tube.
Sample tube: Take 0.1 mL of sample and 0.25 mL of substrate application solution to the 5 mL tube.
Control tube: Take 0.25 mL substrate application solution to the 5 mL tube.

② Add 0.5 mL of buffer solution to each tube and mix fully.

③ Incubate at 37°C for 20 min.

④ Successively add 1 mL of chromogenic agent 1 application solution, 0.5 mL of acid reagent, 0.25 mL of protein precipitator, 0.5 mL of chromogenic agent 2 application solution.

⑤ Add 0.1 mL of sample to control tube.

⑤ Mix fully and centrifuge at 2325×g for 10 min, then take the

supernatant.

⑥ Set the spectrophotometer to zero with blank tube and measure the OD values of each tube at 520 nm with 1 cm optical path cuvette.

CalcμLation

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.

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

2. Tissue and cell 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.

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

3. Whole blood sample:

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

$$\underset{\text{(U/mL)}}{\text{CHE activity}} = \frac{A_1 - A_2}{A_1} \times C \times \frac{V_1}{V_3} \times f$$

[Note]

A_1: The OD value of control tube.

A_2: The OD value of sample tube.

c: the concentration of control tube, 8 μmol/mL.

f: Dilution factor of sample before tested.

V_1: The volume of substrate application solution (0.25 mL)

V_2: The volume of serum and tissue added to the reaction (0.05 mL)

V_3: The volume of whool blood added to the reaction (0.1 mL)

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

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)	2.60	46.50	133.00
%CV	4.2	3.6	3.3

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)	2.60	46.50	133.00
%CV	9.5	9.1	9.6

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%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (U/mL)	15.6	66.7	142.5
Observed Conc. (U/mL)	15.9	70.7	148.2
recovery rate(%)	102	106	104

Sensitivity

The analytical sensitivity of the assay is 1.17 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

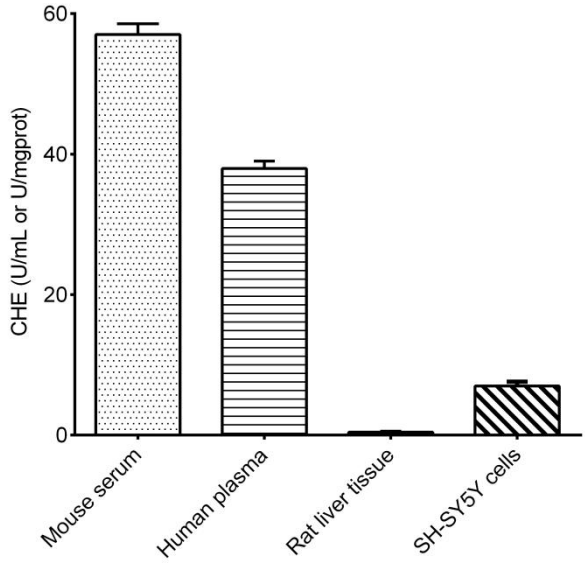
Example analysis：

Dilute mouse serum with normal saline (0.9% NaCl) for 2 times, take 0.05 mL of diluted sample and carry the assay according to the operation steps. The results are as follows:

The average OD value of the sample is 0.208, the average OD value of the control is 0.725, and the calculation result is:

$$\underset{\text{(U/mL)}}{\text{CHE activity}} = \frac{0.725\text{-}0.208}{0.725} \times 8 \times \frac{0.25}{0.05} \times 2 = 57.04\ \text{U/mL}$$

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.