Cholinesterase (CHE) Activity Assay Kit

Catalog No: E-BC-K052-S

Method: Colorimetric method

Specification: 100Assays (Can detect 96 samples without duplication)

Measuring instrument: Spectrophotometer

Sensitivity: 1.17 U/mL

Detection range: 1.17-160 U/mL

- 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 for detection of cholinesterase (ChE) activity in whole blood, serum, plasma, tissue and cell samples.

▲Background

In the body, the main type of cholinesterase (ChE) is acetylcholinesterase (AChE), which is mainly found in the brain and red blood cell membranes. The other type is butyrylcholinesterase (BChE), which is mainly found in plasma. Both forms differ in genetics, structure, and dynamics. ChE is involved in the pathogenesis of some neurodegenerative and related diseases.

▲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	Specification	Storage
Reagent 1	Buffer Solution	60 mL × 2 vials	2-8℃ , 6 months
Reagent 2	Substrate	Powder × 2 vials	2-8℃ , 6 months, shading light
Reagent 3	Diluent 1	10 mL × 1 vial	2-8℃ , 6 months
Reagent 4	Chromogenic Agent 1	Powder × 1 vial	2-8℃ , 6 months
Reagent 5	Alkali Reagent	60 mL × 1 vial	2-8℃ , 6 months
Reagent 6	Acid Reagent	60 mL × 1 vial	2-8℃ , 6 months
Reagent 7	Protein Precipitator	40 mL × 1 vial	2-8℃ , 6 months
Reagent 8	Chromogenic Agent 2	Powder × 1 vial	2-8℃ , 6 months, shading light
Reagent 9	Diluent 2	2 mL × 1 vial	2-8℃ , 6 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.			

▲Materials prepared by users

Instruments

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

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 5 mL)

Reagents

Double distilled water, Normal saline (0.9% NaCl)

▲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 points of the assay

1. The brown-red iron complex after reaction is unstable, and the measurement of OD values must be completed within 20 minutes.
2. Prevent the formulation of bubbles when measuring the OD value, otherwise the OD value will be affected.

Pre-assay preparation

▲Reagent preparation

1. Preparation of 80 μmol/mL reagent 2 stock solution

Dissolve 1 vial of reagent 2 powder with 5 mL of reagent 3 and mix fully. Prepare the fresh solution before use and the prepared solution can be stored at 2-8℃ for a week.

2. Preparation of reagent 2 application solution

Dilute the 80 μmol/mL reagent 2 stock solution with reagent 1 for 10 times. Prepare the needed amount of fresh solution before use. The prepared solution can be stored at 2-8℃ for 24 hours.

3. Preparation of reagent 4 stock solution

Dissolve 1 vial of reagent 4 powder with 60 mL of double distilled water and mix fully. The prepared solution can be stored at 2-8℃ for 3 months.

4. Preparation of reagent 4 application solution

Dilute reagent 4 stock solution with reagent 5 at a ratio of 1:1. Prepare the needed amount of fresh solution before use. The prepared solution can be stored at 2-8℃ for 24 hours.

5. Preparation of reagent 9 application solution

Dilute the reagent 9 with double distilled water at a ratio of 1:39 and mix fully. The prepared solution can be stored at 2- 8℃ for 6 months.

6. Preparation of reagent 8 application solution

Dissolve 1 vial of reagent 8 powder with 60 mL of reagent 9 application solution and mix fully. The prepared solution can be stored at 2-8℃ with shading light for 3 months.

▲Sample preparation

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

▲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 (1.17-160 U/mL).

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
10% Mouse brain tissue homogenate	1

Note: The diluent is normal saline (0.9% NaCl).

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	520 nm

Instructions for the use of transferpettor

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Operating steps

1. For serum (plasma), tissue and cells samples

1) Blank tube: Take 0.3 mL of double distilled water to the 5 mL tube.
   Control tube: Take 0.05 mL of double distilled water and 0.25 mL of reagent 2 application solution to the 5 mL tube.
   Sample tube: Take 0.05 mL of sample and 0.25 mL of reagent 2 application solution to the 5 mL tube.
2) Add 0.5 mL of reagent 1 to each tube and mix fully.
3) Incubate at 37°C for 20 min.
4) Successively add 1 mL of reagent 4 application solution, 0.5 mL of reagent 6, 0.25 mL of reagent 7, 0.5 mL of reagent 8 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.

2. For whole blood samples

1) Blank tube: Take 0.35 mL of double distilled water to the 5 mL tube.
   Control tube: Take 0.25 mL of reagent 2 application solution to the 5 mL tube.
   Sample tube: Take 0.1 mL of sample and 0.25 mL of reagent 2 application solution to the 5 mL tube.
2) Add 0.5 mL of reagent 1 to each tube and mix fully.
3) Incubate at 37°C for 20 min.
4) Successively add 1 mL of reagent 4 application solution, 0.5 mL of reagent 6, 0.25 mL of reagent 7, 0.5 mL of reagent 8 application solution.
5) Add 0.1 mL of sample to control tube.
6) Mix fully and centrifuge at 2325 g for 10 min, then take the supernatant.
7) 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.

▲Operation table

1. For serum (plasma), tissue and cells samples

	Blank tube	Control tube	Sample tube
Sample (mL)			0.05
Double distilled water (mL)	0.3	0.05	
Reagent 2 application solution (mL)		0.25	0.25
Reagent 1 (mL)	0.5	0.5	0.5
Mix fully, incubate at 37°C for 20 min.			
Reagent 4 application solution (mL)	1	1	1
Reagent 6 (mL)	0.5	0.5	0.5
Reagent 7 (mL)	0.25	0.25	0.25
Reagent 8 application solution (mL)	0.5	0.5	0.5
Mix fully, then centrifuge at 2325 g for 10 min, then take the supernatant. 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.			

2. For whole blood samples

	Blank tube	Control tube	Sample tube
Sample (mL)			0.1
Double distilled water (mL)	0.35		
Reagent 2 application solution (mL)		0.25	0.25
Reagent 1 (mL)	0.5	0.5	0.5
Mix fully, incubate at 37°C for 20 min.			
Reagent 4 application solution (mL)	1	1	1
Reagent 6 (mL)	0.5	0.5	0.5
Reagent 7 (mL)	0.25	0.25	0.25
Reagent 8 application solution (mL)	0.5	0.5	0.5
Sample (mL)		0.1	
Mix fully, then centrifuge at 2325 g for 10 min, then take the supernatant. 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.			

▲Calculation

1. Serum (plasma) samples

Definition: The amount of ChE in 1 mL of serum or plasma that react with substrate in 20 minute at 37℃ and decompose 1 μmol acetylcholine is defined as 1 unit.

$$\text{ChE activity (U/mL)} = \frac{A_1\text{-}A_2}{A_1} \times c \times \frac{1\ \text{mL}}{V_1} \times f$$

2. Tissue and cell samples

Definition: The amount of ChE in 1 mL of serum or plasma that react with substrate in 20 minute at 37℃ and decompose 1 μmol acetylcholine is defined as 1 unit.

$$\text{ChE activity (U/mgprot)} = \frac{A_1\text{-}A_2}{A_1} \times c \times \frac{1\ \text{mL}}{V_1} \div C_{pr} \times f$$

3. Whole blood samples

Definition: The amount of ChE in 1 mL of whole blood that react with substrate in 20 minute at 37℃ and decompose 1 μmol acetylcholine is defined as 1 unit.

$$\text{ChE activity (U/mL)} = \frac{A_1\text{-}A_2}{A_1} \times c \times \frac{1\ \text{mL}}{V_2} \times f$$

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 serum (plasma), tissue or cell sample added to the reaction, 0.05 mL.

V_2: The volume of whole blood sample added to the reaction, 0.1 mL.

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

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

Appendix I Performance characteristics			
Detection range	1.17-160 U/mL	Average intra-assay CV (%)	3.7
Sensitivity	1.17 U/mL	Average inter-assay CV (%)	9.4
Average recovery rate (%)	104		

▲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 table. 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:

$$\text{ChE activity (U/mL)} = \frac{0.725-0.208}{0.725} \times 8 \times \frac{1\ \text{mL}}{0.05} \times 2 = 228.19\ \text{U/mL}$$

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Whole blood

Bottom up the heparin anticoagulated whole blood to make it mix fully, then take 0.1 mL of the whole blood and add 0.4 mL of pre-cooled double distilled water. Mix fully for 1 min and stand for 15 min until the prepared hemolysis is transparent when observing under light.

▲ Serum

Collect fresh blood and stand at 25°C for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4°C . 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°C 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°C . 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°C for a month.

▲ Tissue sample

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2 - 8°C . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C . Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M). If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

▲ Cells

Collect the cells (don't use trypsin or EDTA) and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (10^6): homogenization medium (μL) =1: 300-500. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M). If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

Note:

1. Homogenized medium: Normal saline (0.9% NaCl).

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.)

(3) Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2 s/time, interval for 3 s, the total time is 5 min).

▲ Note for sample

1. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
2. If a lysis buffer is used to prepare tissue or cell homogenates, there is a possibility of causing a deviation due to the introduced chemical substance.