

PRODUCT INFORMATION & MANUAL

Cholinesterase Activity Assay Kit (Colorimetric) NBP3-25928

For research use only.

Not for diagnostic or therapeutic procedures.

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Cholinesterase Activity Assay Kit (Colorimetric)

Catalog No: NBP3-25928

Method: Colorimetric method

Specification: 96T (Can detect 92 samples without duplication)

Instrument: Microplate reader

Sensitivity: 2.17 U/mL

Detection range: 2.17-71.33 U/mL

Average intra-assay CV (%): 2.4

Average inter-assay CV (%): 6.4

Average recovery rate (%): 102

- ▲ 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 for detection of cholinesterase (ChE) activity in serum (plasma) and animal tissue 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 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	30 mL × 1 vial	2-8°C , 12 months
Reagent 2	Substrate	Powder × 2 vials	2-8°C , 12 months, shading light
Reagent 3	Diluent A	1.5 mL × 2 vials	2-8℃ , 12 months
Reagent 4	Chromogenic Agent A	Powder × 1 vial	2-8℃ , 12 months
Reagent 5	Alkaline Reagent	20 mL × 1 vial	2-8℃ , 12 months
Reagent 6	Acid Reagent	24 mL × 1 vial	2-8°C , 12 months
Reagent 7	Protein Precipitator	20 mL × 1 vial	2-8℃ , 12 months
Reagent 8	Chromogenic Agent B	Powder × 1 vial	2-8°C , 12 months, shading light
Reagent 9	Diluent B	1 mL × 1 vial	2-8℃ , 12 months
	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



1 Instruments

Test tube, centrifuge, Vortex mixer, 37°C water bath, Microplate reader (505-535 nm, optimum wavelength: 520 nm)



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.

A 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 colorimetry must be completed within 20 minutes.
- 2. There should be no bubbles in the wells of the microplate when measuring the OD value.

Pre-assay preparation

▲ Reagent preparation

- 1. Bring all reagents to room temperature before use.
- 2. Preparation of 80 µmol/mL reagent 2 stock solution:

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

3. Preparation of reagent 2 application solution:

Dilute the 80 µmol/mL reagent 2 stock solution with reagent 1 at a ratio of 1:9. Prepare the needed amount of fresh solution before use. The prepared solution can be stored at 2-8°C for 24 hours.

4. Preparation of reagent 4 stock solution:

Dissolve 1 vial of reagent 4 powder with 20 mL of double distilled water and mix fully. The prepared solution can be stored at 2-8°C for 3 months.

5. 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°C for 24 hours.

6. 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°C for 6 months.

7. Preparation of reagent 8 application solution:

Dissolve 1 vial of reagent 8 powder with 20 mL of reagent 9 application solution and mix fully. The prepared solution can be stored at 2-8°C for 3 months with shading light.

▲ Sample preparation

1. Serum and plasma samples:

Detect the sample directly.

2. Tissue sample:

Accurately weigh the tissue, add 9 times the volume of normal saline (0.9% NaCl) according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 10000 g for 10 min. If the supernatant is turbidity after centrifugation, centrifuge the supernatant repeated before use. Meanwhile, determine the protein concentration of supernatant.

▲ 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 (2.17-71.33 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
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).

Assay protocol

▲ Plate set up

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

Note: A, blank wells; B, control wells; S1-S92, sample wells.

▲ Detailed operation steps

2. The measurement of samples

(1) Blank tube: Take 80 uL of double distilled water to the 2 mL EP tube.

Control tube: Take 20 uL of double distilled water and 60 uL of reagent 2 application solution to the 2 mL EP tube.

Sample tube: Take 20 uL of sample and 60 uL of reagent 2 application solution to the 2 mL EP tube.

- (2) Add 150 uL of reagent 1 to each tube.
- (3) Mix fully and incubate at 37°C for 20 min.
- (4) Successively add 200 uL of reagent 4 application solution, 150 uL of reagent 6, 100 uL of reagent 7, 100 uL of reagent 8 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.

▲Summary operation table

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	Blank tube	Control tube	Sample tube			
Sample (µL)			20			
Double distilled water (µL)	80	20				
Reagent 2 application solution (µL)		60	60			
Reagent 1 (µL)	150	150	80			
Mix fully and incubate at 37℃ for 20 min.						
Reagent 4 application solution (µL)	200	200	200			
Reagent 6 (µL)	150	150	150			
Reagent 7 (µL)	100	100	100			
Reagent 8 application solution (µL)	100	100	100			
Contribute at 2200 g for 10 min. Take 250 ut of superpotent to the						

Centrifuge at 2300 g for 10 min. Take 250 µL of supernatant to the corresponding wells of microplate, measure the OD value of each well.

▲ Calculation

1. Serum/plasma sample:

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.

CHE activity (U/mL) =
$$(\Delta A_1 - \Delta A_2) \div \Delta A_1 \times C \times V_1 \div V_2 \times f$$

2. Tissue:

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.

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

Note:

 $\Delta A_1 : OD_{Control} - OD_{Blank.}$

 $\Delta A_2: OD_{Sample} - OD_{Blank.}$

C: The concentration of reagent 2 application solution, 8 µmol/mL.

 V_1 : The volume of reagent 2 application solution, 0.06 mL.

V₂: 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

▲ Example analysis

For human plasma, dilute for 2 times, and carry the assay according to the operation table.

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) = $((0.426 - 0.044) - (0.236 - 0.044)) \div (0.426 - 0.044) \times 8 \times 0.06 \div 0.02 \times 2 = 23.85 \text{ U/mL}$

Appendix II References

- 1. Giacobini E. Cholinesterases: New Roles in Brain Function and in Alzheimer's Disease[J]. Neurochemical Research, 2003, 28(3-4): 515-522.
- 2. Deutsch S I, Campbell M. Status of cholinesterase activities in blood in neuropsychiatric disorders[J]. Neurochemical Research, 1984, 9(7): 863-869.