PRODUCT INFORMATION & MANUAL

Acetylcholinesterase/ ACHE Activity Assay Kit (Colorimetric)

NBP3-25835

For research use only.
Not for diagnostic or therapeutic procedures.

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Novus kits are guaranteed for 6 months from date of receipt

Acetylcholinesterase/ACHE Activity Assay Kit (Colorimetric)

Catalog No: NBP3-25835
Method: Colorimetric method
Specification: 50Assays **(**Can detect 24 samples without duplication)
Measuring instrument: Spectrophotometer
Sensitivity: 0.01 U/mL
Detection range: 0.01-5.0 U/mL

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.

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

Application

This kit can be used for detection of Acetylcholinesterase/ACHE activity in animal tissue, serum (plasma), whole blood and cultured cells, and cell culture supernatant.

Detection significance

Acetylcholinesterase also known as ACHE or acetylhydrolase, is the primary cholinesterase in the body. It is an enzyme that catalyzes the breakdown of acetylcholine and of some other choline esters that function as neurotransmitters. ACHE is found at mainly neuromuscular junctions and in chemical synapses of the cholinergic type, where its activity serves to terminate synaptic transmission. It belongs to carboxylesterase family of enzymes. It is the primary target of inhibition by organophosphorus compounds such as nerve agents and pesticides.

Detection principle

Acetylcholinesterase hydrolysis acetylcholine (Ach) to produce choline and acetic acid. Then choline reacts with sulfydryl substrate to form TNB (Sym-Trinitrobenzene), a yellow compound which can be measured at 412 nm. And ACHE activity can be calculated indirectly.

Kit component

	Item	**Specification**	**Storage**
Reagent 1	Standard	Powder ×3 vials	2-8℃, 12 months
Preparation of 1 μmol/mL standard application solution: dissolve a vial of reagent 1 powder with 10 mL of normal saline and mix fully. Prepare fresh solution before use.			
Reagent 2	Substrate	Powder ×2 vials	2-8℃, 12 months
Substrate buffer: dissolve a vial of reagent 2 powder with 20 mL of normal saline and mix fully. The prepared substrate buffer can be stored at 4℃ for 2 weeks.			
Reagent 3	Chromogenic Agent Stock Solution	3 mL ×1 vial	2-8℃, 12 months, shading light
Chromogenic agent application solution: Dilute the stock solution with normal saline at 1:9. Prepare the needed amount before use. The prepared solution can be stored at 2-8℃ with shading light.			
Reagent 4	Inhibitor	2 mL ×1 vial	Room temperature, 12 months
	Empty Plastic Bottle	1 vial	Room temperature, 12 months
After opened, please store the reagent 4 in the empty bottle provided in the kit.			
Reagent 5	Transparent Agent	6 mL ×1 vial	Room temperature, 12 months
Reagent 5 may form sediment or turbidity in cold weather. It should be incubated in 37℃ water bath to transparent before use.			
Reagent 6	Normal Saline	60 mL ×2 vials	Room temperature, 12 months

Experimental instrument

Test tube, Micropipettor, Vortex mixer, 37℃water bath, Spectrophotometer (412 nm)

The preparation of samples

1. **Tissue sample:**
   Weigh the tissue accurately, add 9 times the volume of normal saline according to Weigh (g): Volume (mL) = 1:9. Make the mechanical homogenate on ice. Centrifuge at 2500 rpm for 10 min, then take the supernatant for detection. Meanwhile, determine the concentration of supernatant.
2. **Serum (plasma):**
   Take whole blood and centrifuge at 1000~1500 rpm for 8 min, then take the upper layer serum (plasma). Dilute the serum or plasma with normal saline at 1:9 for detection (the specific dilution ratio should be determined according to the pre-experiment).
3. **Whole blood:**
   Take 0.1 mL of whole blood and add double distilled water to 10 mL (1:99 dilution), mix fully. The sampling volume can be reduced if your sample amount is little. Take a mL (generally 0.1 mL) of sample for detection. Each sample should be mixed fully before sampling.

Operation steps

	Sample tube	Control tube	Standard tube	Blank tube
Sample (mL)	a*			
1 μmol/mL Standard application solution (mL)			a*	
Double distilled water (mL)				a*
Substrate buffer (mL)	0.5	0.5	0.5	0.5
Chromogenic agent application solution (mL)	0.5	0.5	0.5	0.5
Mix fully, incubate for exactly 6 min at 37℃.				
Inhibitor reagent (mL)	0.03	0.03	0.03	0.03
Transparent agent (mL)	0.1	0.1	0.1	0.1
Sample (mL)		a*		
Mix fully and stand for 15 min. Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 412 nm with 0.5 cm optical path cuvette.				

[Note]

(1) The control tube should be set for every sample, because the absorbance difference of control tube for each sample is large.

(2) Stand the tubes at room temperature for 15 min before the colorimetric detection. There may be sediment or turbidity if the room temperature is too low. Incubate the tube in 37℃ water bath for a while until the solution turns clear and detection. This will not affect the experiment result.

(3) The reaction time is short, so the sample number cannot be too many in batch detection. The reaction time should be accurately controlled, otherwise the accuracy of experiment will be affected.

(4) a* presents the applied volume of sample, standard and double distilled water.

a) Dilute serum (plasma) for 10 times with normal saline before detection. The reference volume is 30~50 μL.

b) The reference volume for 10% brain tissue homogenate is 30~50 μL.

c) Take 0.1 mL of whole blood diluent (diluted at 1:99). Mix fully before sampling.

Calculation of results

1. For tissue sample

Definition: The enzyme amount of ACHE in 1 mg of tissue protein that hydrolyze 1 μmol of substrate in 6 minutes at 37℃ is defined as 1 unit.

A-CHE activity in tissue (*U*/*mgprot*)

$= \frac{OD_{Sample} - OD_{Control}}{OD_{Standard} - OD_{Blank}} \times$ Concentration of standard (1 $\mu mol/mL$)

$\div$ Protein concentration of sample ($mgprot/mL$)

2. For serum (plasma) sample

Definition: The enzyme amount of ACHE in 1 mL of sample that hydrolyze 1 μmol of substrate in 6 minutes at 37℃ is defined as 1 unit.

A-CHE activity in serum (*U/mL*)

$= \frac{OD_{Sample} - OD_{Control}}{OD_{Standard} - OD_{Blank}} \times$ Concentration of standard (1 $\mu mol/mL$)

$\times$ Dilution factor of sample before tested

3. For whole blood sample

Definition: The enzyme amount of ACHE in 1 mL of whole blood sample that hydrolyze 1 μmol of substrate in 6 minutes at 37℃ is defined as 1 unit.

A-CHE activity in whole blood (*U/mL*)

$= \frac{OD_{Sample} - OD_{Control}}{OD_{Standard} - OD_{Blank}} \times$ Concentration of standard (1 $\mu mol/mL$)

$\times$ Dilution factor of sample before tested

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 the kit is 12 months.

4. Do not use components from different batches of kit.