Acetylcholinesterase (A-CHE) Assay Kit

Catalog No: SH0060
Method: Colorimetric method
Specification: 50 tubes/ 24 samples

Experimental instrument

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

Application

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

Kit component

	Item	Specification	Storage
Reagent 1	Standard	Powder, 3 vials	4℃, 6 months
	Preparation of 1 μml/mL Standard application solution: Add 10 mL of normal saline into 1 vial of standard and mix fully for use. Prepare fresh solution before use.		
Reagent 2	Substrate	Powder, 2 vials	4℃, 6 months
	Preparation of Substrate buffer: Add 10 mL of normal saline into 1 vial of **Reagent 2 Powder**, mix fully. Thee prepared Substrate buffer can be stored at 4℃ for 2 weeks.		
Reagent 3	Chromogenic agent stock solution	Liquid, 3 mL×1 vials	-20℃, 6 months
	Preparation of Chromogenic agent application solution: Dilute the stock solution with normal saline at 1:9. Prepare the needed amount. Or prepare 30 mL at one time and store it at 4℃ in the dark.		
Reagent 4	Inhibitor	Liquid, 2 mL ×1 vial	Room temperature, 6 months
Reagent 5	Coaggulating reagent	Liquid, 6 mL ×1 vial	Room temperature, 6 months
Reagent 5 may form sediment or turbidity in cold weather. It should be incubated in 37℃ water bath to transparent before use.			
Normal saline	60 mL ×2 vials		Room temperature, 6 months
Preparation of Working solution: Reagent 1: Reagent 2 application solution: Reagent 3 application solution= 50: 1: 1. Prepare fresh solution and according to the needed amount before use.			

501 Changsheng S Rd, Nanhu Dist, Jiaxing, Zhejiang, China

Tel: 86 21 31007137 | E-mail: save@bt-laboratory.com | www.bt-laboratory.com

Sample treatment

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 mechanic homogenate in ice. Centrifuge at 2500 rpm for 10 min, then take the supernatant for detection. (Take part of the supernatant to detect the protein concentration.)

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 conventional detection (the specific dilution ration 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 1 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*			
1μmol/mL Standard working solution (mL)			a*	
Double distilled water				a*
Substrate buffer (mL)	0.5	0.5	0.5	0.5
Color-developing reagent (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 reagent(mL)	0.1	0.1	0.1	0.1
Sample (mL)		a*		10

Mix fully and stand for 15 min. Measure the OD values of each tube (412 nm wavelength, cuvette of 1cm diameter, and set to zero with double distilled water).

[Note]

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

(2) Place the tube 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. ACH-E activity in tissue (*U/mgprot*)

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

   ÷ Protein concentration of sample（mg[illegible]$/mL$)

2. ACH-E activity in serum (*U/mL*)

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

   × Dilution factor of sample before tested

Advantages

1. High sensitivity. The ACH-E activity can be detected with micro-amount sample.
2. Typing is operability.
3. Ease of use.
4. Accurate and stable.
5. No need of advanced instruments such as HPLC. Normal spectrophotometer is enough for this experiment.

This manual must be read attentively and completely before using this product.
May you have any problems, please contact our Technical Service Center for help.
Phone: 86-21-3100-7137
Email: save@bt-laboratory.com
Website: www.bt-laboratory.com