Acetylcholinesterase assay kit

(Cat.No:BC096 Size:50T/24S Colorimetric Method)

1. Composition & Preparation

Reagent 1: Standard powder×3 vials, can be stored at 4℃ for 6 months.

1μmol/ml standard working solution: Take 1 vial standard powder, add 10ml physiological saline, mix sufficiently, this working solution should be used soon after preparation.

Reagent 2: Substrate powder×2 vials, can be stored at 4℃ for 6 months. When use, add 20ml physiogical saline in each vial, prepared as substrate buffer, can be stored at 4℃ for 2 weeks.

Reagent 3: Chromogenic agent stock solution, 3ml×1 vial, can be stored at 4℃ for 6 months. When use, dilute stock solution with physiological saline at ratio of 1:9 to prepare chromogenic agent working solution, how much you need, how much you prepare. You can also prepare 30ml working solution once, it can be stored by cold preservation away from light at 4℃ for 3 months.

Reagent 4: Inhibitor, liquid 2ml×1 vial. After open, it should be contained in a small plastic bottle (you can find this bottle in kit), can be stored at 4℃ for 6 months.

Reagent 5: Clarificant, liquid 6ml×1 vial, can be stored at room temperature for 6 months. Sediment or turbidity may appear in cold days, please place it in 37℃ water bath until it becomes limpid.

Physiological saline: 60ml×2 bottles, can be stored at room temperature for 6 months.

2. Operation Procedure

（1）Sample pretreatment:

① **Tissue homogenate preparation:** Weigh tissue accurately, add 9 times volume physiological saline to prepare 10% homogenate, mechanical homogenization under ice-water bath conditions, centrifugate at

2500rpm for 10 minutes, take supernatant to assay. Please measure protein concentration at the same time, you can dilute homogenate to 1~2% to measure protein concentration by Coomassie brilliant blue.

② **Blood serum (or plasma):** Take unanticoagulated (or anticoagulated) whole, centrifugate at 1000~1500rpm for 8 minutes, take upper layer ofblood serum (or plasma). When use, dilute blood serum (or plasma) with physiological saline at ratio of 1:9.

③ **Whole blood:** Take 0.1ml anticoagulated whole blood, add double distilled water until volume reaches to 10ml (1:99 dilution), mix thoroughly. If your sample volume is too small, then you can decrease sampling volume but keep the ratio. For example, you can take 0.01ml or 0.02ml anticoagulated whole blood and add double distilled water until volume reaches to 1ml or 2ml. Take **a**ml (0.1ml in general) to assay, please mix each sample sufficiently before sampling.

(2) Operation table:

	Assay	**Contrast**	**Standard**	**Blank**
Sample (ml)	**a** *			
1μmol/ml standard working solution (ml)			**a** *	
Distilled water (ml)				**a** *
Substrate buffer (ml)	0.5	0.5	0.5	0.5
Colorimetric application solution (ml)	0.5	0.5	0.5	0.5
Mix, incubate at 37℃ for exactly 6 minutes				
Inhibitor（ml）	0.03	0.03	0.03	0.03
Clarificant (ml)	0.1	0.1	0.1	0.1
Sample (ml)		**a** *		
Mix sufficiently, place for 15 minutes at room temperature (18-25℃), transfer in cuvettes of 0.5cm light path, measure OD values of all tubes at 412nm (adjust zero by distilled water).(Or, draw 200 μL of the reaction solution from each tube and add it to a 96-well plate. Read the values at 412 nm using a microplate reader.)				

Note 1: You must make contrast tube for each sample, because there may be large variance between contrast tube OD values of different sample.

Note 2: Place test tubes at room temperature for 15 minutes before colorimetric analysis. If room temperature is too low, sediment or turbidity may appear, in this situation, please place test tube in 37℃ water bath until solution becomes clear. Then you can start measurement, it won't affect reasult.

Note 3: Because reaction time is quite short, so when you do assay in batches, don't put too many samples in one batch, please control reaction time length accurately or it will affect experimental accuracy.

Note 4: a* is referenced volume of sample, standard, and distilled water taken, all of which are equal:

- 10 times diluted (by physiological saline) blood serum (or plasma): 30~50μl
- 10% brain tissue homogenate: 10~50μL
- 1:99 diluted whole blood: 0.1mL (mix sufficiently before taking sample).

3. Calculation

（1）Tissue homogenate AChE Activity:

① **Definition:** React at 37℃ for 6 minutes**,** 1μmol substrate decomposing in hydrolysis reaction system per mg tissue protein is considered as 1 ACHE activity unit (U).

② **Formula:**

$$\text{AChE Activity (U/mgprot)} = \frac{OD_{Sample} - OD_{Control}}{OD_{Standard} - OD_{Blank}} \times C_{Standard} \div C_{pr}$$

$C_{standard}$: Standard concentration, 1μmol/mL;

Cpr:protein concentration of tissue homogenate (mgprot/mL)

③ **Examples:**

a. 0.03 mL of 10% rat brain tissue homogenate was used to measure acetylcholinesterase activity. The OD values were: test tube 0.283, control

tube 0.080, standard tube 0.192, and blank tube 0.034. The standard tube concentration was 1 μmol/mL. The protein concentration of 10% rat brain tissue homogenate was 3.84 mg prot/mL. The calculated results are:

$$\text{Tissue ACHE activity (U/mgprot)} = \frac{0.283 - 0.080}{0.192 - 0.034} \times 1 \div 3.84 = 0.3346\text{U/mgprot}$$

b. 0.03 mL of 10% Japanese shrimp muscle homogenate was used to measure acetylcholinesterase activity. The OD values were: test tube 0.193, control tube 0.054, standard tube 0.195, and blank tube 0.040. The standard tube concentration was 1 μmol/mL. The protein concentration of 10% Japanese shrimp muscle tissue homogenate was 6.2638 mg prot/mL. The calculation result is as follows:

$$\text{Tissue ACHE activity (U/mgprot)} = \frac{0.193 - 0.054}{0.195 - 0.040} \times 1 \div 6.2638 = 0.1432\text{U/mgprot}$$

c. 0.03 mL of 10% Chinese sturgeon brain tissue homogenate was used to measure acetylcholinesterase activity. The measured OD values were: test tube 0.308, control tube 0.076, standard tube 0.204, and blank tube 0.048. The standard tube concentration was 1 μmol/mL, and the protein concentration of 10% Chinese sturgeon brain tissue homogenate was 4.0698 mg prot/mL. The calculation result is as follows:

$$\text{Tissue ACHE activity (U/mgprot)} = \frac{0.308 - 0.076}{0.204 - 0.048} \times 1 \div 4.0698 = 0.3654\text{U/mgprot}$$

(2) Blood serum AChE:

① **Definition:** React at 37℃ for 6 minutes, 1μmol substrate decomposing in hydrolysis reaction system per ml blood serum is considered as 1 TCHE activity unit (U).

② **Formula:**

$$\text{Serum (plasma) AChE Activity (U/mL)} = \frac{OD_{Sample} - OD_{Control}}{OD_{Standard} - OD_{Blank}} \times C_{Standard} \times N$$

$C_{standard}$: Standard concentration, 1μmol/mL;

N: Dilution factor of the sample before testing.

③ Example:

0.03 mL of serum diluted 10-fold was used to measure acetylcholinesterase activity. The OD values were: test tube 0.498, control tube 0.086, standard tube 0.190, and blank tube 0.032. The standard tube concentration was 1 μmol/mL. The calculated results are as follows:

$$\text{Blood serum ACHE activity (U/mL)} = \frac{0.498 - 0.086}{0.190 - 0.032} \times 1 \times 10 = 26.08 \text{U/mL}$$

(3) Whole blood ACHE:

① Definition: React at 37℃ for 6 minutes, 1μmol substrate decomposing in hydrolysis reaction system per ml whole blood is considered as 1 TCHE activity unit (U).

② Formula:

$$\text{Whole blood AChE Activity (U/mL)} = \frac{OD_{Sample} - OD_{Control}}{OD_{Standard} - OD_{Blank}} \times C_{Standard} \times N$$

$C_{standard}$: Standard concentration, 1μmol/mL;

N: Dilution factor of the sample before testing.

③ Example:

Take 0.1 mL of a 1:99 diluted whole blood solution to measure acetylcholinesterase activity. The OD values were: test tube 0.492, control tube 0.212, standard tube 0.364, and blank tube 0.040. The standard tube

concentration was 1 μmol/mL. The calculated results are:

$$\text{Whole blood ACHE activity（U/mL）} = \frac{0.492 - 0.212}{0.364 - 0.040} \times 1 \times 100 = 86.42\text{U/mL}$$

4. Assay significance

ACHE is hydrolase of cholinergic neurotransmitter acetylcholine, it participates in various important functions such as autonomic nerve function regulation, brain thinking, memory,etc. T-CHE activity change follows organ function change. Phosphate pesticide is specific inhibitor of T-CHE, it decreases T-CHE activity after entering body from various pathways.

5. Assay principle

T-CHE hydrolyzes acetylcholine to produce choline & acetic acid. Choline can react with sulfydryl chromogenic agent to produce sym-trinitrobenzene yellow compound. It is able to calculate T-CHE activity by measuring OD values of produced choline.

6. Advantages

①、 High sensitivity, ACHE activity in microscale samples can be also measured.

②、Can be classified. There is integrated assay kit to measure B-TCHE.

③、Convenient, rapid, accurate & stable.

④、 Do not require advanced equipments such as gas chromatograph, etc. Common spectrophotometer is enough.