Butyrocholinesterase (BCHE) Assay Kit

（Cat/No.:BC121　　Size: 50T/24S）

1. Assay principle

Butyrylcholinesterase (BChE) catalyzes the hydrolysis of butyrylcholine into choline and acetic acid. Choline reacts with a thiol chromogenic reagent to form a TNB yellow compound, which is quantified by colorimetry based on color intensity. The amount of choline (the hydrolysis product) reflects the activity of butyrylcholinesterase.

2. Composition & Preparation (The kit is valid for 3 months)

Reagent 1：Standard working solution ,powder×3 vialsstore at 4℃. Take one vial of standard, add 10 mL of physiological saline, mix well to prepare a **1 μmol/mL standard working solution**. Prepare fresh before use.

Reagent 2： Substrate powder×2 vials. For each vial, add 20 mL of physiological saline. Prepare immediately as needed. Store at 4℃ for two weeks after preparation.

Reagent 3： Chromogenic agent stock solution, 3 mL per bottle, stored at 4℃. Dilute with normal saline at a ratio of 1:9 during use to prepare the color development application solution. The amount to be prepared depends on the need. It can also be prepared in a total volume of 30 mL at once. Store in the dark and at low temperature for up to 3 months.

Reagent 4: Inhibitor, liquid × 2 bottles, stored at room temperature.

Reagent 5: Clearing agent, liquid, 6 mL per bottle, store at room temperature.

Sodium Chloride Solution: 60mL×2 bottle, store at room temperature.

3. Required instruments and reagents

Spectrophotometer adjustable to 412 nm with 0.5 cm light path cuvettes (or microplate reader with 96-well plates), 37℃ water bath (or incubator), double-distilled water (or distilled water), vortex mixer, protein assay reagent (available from our company).

4. Operating Procedure

1.Tissue Sample Pretreatment：

Tissue Sample: Weigh the tissue accurately, add 9 volumes of physiological saline at a weight (g):volume (mL) ratio of 1:9, mechanically homogenize in an ice-water bath, centrifuge at 2500 rpm for 10 minutes, and collect the supernatant (10% homogenate supernatant).

Serum Sample： Take anticoagulated whole blood, centrifuge at 1000–1500 rpm for 10 minutes, and collect the upper plasma layer. Serum can also be used; for detection, dilute the serum (or plasma) 1:19 with physiological saline.

Whole Blood Sample： Take 0.1 mL anticoagulated whole blood, add physiological saline to a final volume of 10 mL (1:99 dilution), then take a mL (typically 0.1 mL) for detection. Mix each sample thoroughly before sampling.

2.Operation steps:

	Blank	Standard	Sample	Contrast
Sample (mL)			a	
1 μmol/mL standard solution application liquid (mL)		a		
Deionized water (mL)	a			
Substrate buffer solution (mL)	0.5	0.5	0.5	0.5
Color development application liquid (mL)	0.5	0.5	0.5	0.5
Mix well and allow reaction at 37°C for exactly 6 minutes.				
Inhibitor (mL)	0.03	0.03	0.03	0.03
Transparent agent (mL)	0.1	0.1	0.1	0.1
Sample (mL)				a
Mix thoroughly, stand for 15 min, set wavelength to 412 nm (0.5 cm light path), zero with double-distilled water, and measure the absorbance (A) of each tube using a spectrophotometer (or take 200 μL from each tube into a 96-well plate and read with a microplate reader at 412 nm).				

Note: a in the table above refers to the recommended sample volume: (1) Rat serum should be 20-fold diluted with physiological saline before testing, with a recommended sample volume of 30–50 μL; (2) The recommended sample volume for 10% brain tissue homogenate is 30–50 μL; (3) Take 0.1 mL of 1:99 diluted whole blood solution. A control tube must be prepared for each sample, as the absorbance of control tubes varies significantly between samples.

5. Definition and Calculation Formula

1. Butyrylcholinesterase (BChE) in Tissue Homogenate Calculation

Definition: Each milligram of tissue protein sample was incubated at 37℃ for 6 minutes. In the hydrolysis reaction system, 1 μmol of the substrate was defined as 1 unit (U).

$$\textbf{B-CHE Activity(U/mgprot)}=\frac{A_{sample}-A_{contrast}}{A_{standard}-A_{blank}}\times C_{standard}\div Cpr$$

2. Butyrylcholinesterase (BChE) in Serum (or Whole Blood Calculation

Definition: Each milliliter of serum (or whole blood) sample is incubated at 37℃ for 6 minutes. In the hydrolysis reaction system, 1 μmol of the substrate represents 1 unit (U).

$$\textbf{B-CHE Activity(U/ml)}=\frac{A_{sample}-A_{contrast}}{A_{standard}-A_{blank}}\times C_{standard}\div N$$

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

Cpr: Tissue Homogenate Protein Concentration: mgprot/mL (prot = protein)

N: Dilution factor of the sample before testing

6. Characteristics of this method

1. High sensitivity: Trace samples can detect butyrylcholinesterase (BCHE) levels. Typing is available; a companion kit is also provided for acetylcholinesterase (ACHE) detection.
2. Simple, rapid, accurate and stable method: No sophisticated instruments such as gas chromatography are required—only a general spectrophotometer is needed.
3. When reading with a microplate reader, avoid bubbles in the microplate wells.
4. If the corresponding wavelength is unavailable, select the nearest one (with a deviation of no more than 10 nm if possible).