Cholinesterase (CHE) Assay Kit

（Cat/No.: BC115　Size:50T/48S）

1. Assay principle (Colorimetry)

Cholinesterase (CHE) in blood and tissues catalyzes the hydrolysis of acetylcholine into choline and acetic acid. The unhydrolyzed acetylcholine reacts with hydroxylamine to form acetylhydroxylamine, which then combines with iron ions in an acidic solution to generate a brown complex. The enzyme activity is calculated based on the intensity of the brown color.

2. Composition & Preparation:(The kit is valid for 6 months)

Reagent 1： Solution 60mL×1 bottle, store at room temperature.

Reagent 2: Acetylcholine powder×1 vial, store at -20℃; Diluent: 5 mL × 1 vial, store at 4℃. **Preparation of 80μmol/mL Acetylcholine Substrate Stock Solution:** Pour 1 vial of acetylcholine powder into 5 mL of diluent to dissolve, and store at -20℃. **Preparation of 8 μ mol/mL Acetylcholine Working Solution: Dilute at a ratio of 80μmol/mL acetylcholine substrate stock solution : reagent one = 1 : 9.** Prepare as needed, using the exact amount required.

Reagent 3: Powder A × 1 vial; Solution B 30 mL × 1 bottle; Solution C 30 mL × 1 bottle.Storage condition: Room temperature. Preparation procedure: Prior to testing, pour the entire contents of Powder A into Solution B and shake thoroughly to mix well. For use, mix the Powder A-Solution B mixture with Solution C in equal volumes (as required) and homogenize completely to prepare **Reagent 3**. Prepare fresh immediately before use.

Reagent 4: Solution 30 mL × 1 bottle. Store at room temperature.

Reagent 5: Solution 20 mL × 1 bottle. Store at room temperature.

Reagent 6: Powder × 1 bottle; Diluent 30 mL × 1 bottle.Store at 4℃ in the dark. Preparation procedure: Prior to testing, dissolve the entire contents of 1 bottle of Powder with 1 bottle of Diluent. Mix thoroughly until completely dissolved, then set aside for use.

3.Required instruments and reagents

三． Visible light spectrophotometer, 1 cm cuvette, 37℃ water bath, centrifuge, vortex mixer, distilled water, protein determination reagent (available from our company).

4. Preparation steps

(1) Measurement of serum (plasma) and tissues:

1. Sample pre-treatment：

Serum (plasma): Direct sampling and testing.

Tissue Sample： Accurately weigh the tissue sample. Add normal saline at a ratio of tissue weight (g) : saline volume (mL) = 1:9, then homogenize the mixture under ice bath condition to prepare a 10% tissue homogenate. Centrifuge the homogenate at 2500 rpm for 10 minutes, and collect the supernatant for subsequent assays. Meanwhile, determine the tissue protein concentration using either the BCA method or Coomassie Brilliant Blue method. Note: Protein Assay Kits are available from our company.

2. Operating Table:

	Blank	Contrast	Sample
Sample to assay (ml)			0.05
Distilled water (ml)	0.30	0.05	
8 umol/ml acetylcholine working solution (ml)		0.25	0.25
Reagent 1 (ml)	0.5	0.5	0.5
Mix sufficiently, place in 37℃ water bath for 20 minutes			
Reagent 3 working solution (ml)	1.0	1.0	1.0
Reagent 4 (ml)	0.5	0.5	0.5
Reagent 5 (ml)	0.25	0.25	0.25
Reagent 6 (ml)	0.5	0.5	0.5
Mix well, centrifuge at 3500 rpm for 10 minutes, then take the supernatant. Measure the absorbance A of each tube at 520 nm with a 1 cm optical path, and zero the blank tube.			

3. Serum Samples Calculation:

Definition: Each milliliter of serum was incubated at 37°C with the substrate for 20 minutes, and 1 mole of acetylcholine was decomposed to 1 unit of activity.

Calculation Formula:

$$\textbf{Blood Serum CHE Activity(U/ml)}=\frac{A_{contrast}-A_{sample}}{A_{contrast}}\times C_{standard}\times V_{standard}\times\frac{1}{V_{sample}}$$

4.Tissue Sample Calculation:

Definition: Each milligram of protein in the tissue, when subjected to the substrate at 37°C for 20 minutes, 1 mole of acetylcholine is decomposed to produce 1 unit of activity.

Calculation Formula:

$$\textbf{Tissue CHE Activity(U/mgprot)}=\frac{A_{contrast}-A_{sample}}{A_{contrast}}\times C_{standard}\times V_{standard}\times\frac{1}{V_{sample}\times Cpr}$$

$C_{standard}$: Acetylcholine concentration: 8 mol/mL;
$V_{standard}$: Acetylcholine Volume: 0.25 mL
V_{sample}: Tissue Sample Volume: 0.05 mL
Cpr： Tissue Homogenate Protein Concentration: mgprot/mL (prot = protein)

5. Calculation Example

Example 1: Take the rat serum and follow the operation procedures. The OD value of the control tube was measured to be 0.360, and the OD value of the test tube was 0.203. The calculation results are as follows:

$$\textbf{Rat Serum CHE Activity(U/ml)}=\frac{0.360-0.203}{0.360}\times 8\times 0.25\times\frac{1}{0.05}=17.45\ U/ml$$

Example 2： Take 10% rat brain homogenate and follow the operation procedure. The OD value of the control tube was measured to be 0.360, and the OD value of the test tube was 0.214. At the same time, the protein concentration of 10% rat brain homogenate was measured to be 5.74 mg/mL. Then, the calculation is as follows:

$$\textbf{Tissue CHE Activity(U/mgprot)}=\frac{0.360-0.214}{0.360}\times 8\times 0.25\times\frac{1}{0.05}\div 5.74=2.83\ U/mgprot$$

6. Measurement of whole blood

1. **Tissue Sample Pretreatment:** Gently invert the heparin-anticoagulated whole blood to mix evenly, then aspirate 0.1 mL. Mix with 0.4 mL of cold distilled water, vortex thoroughly for 1 minute, and stand for 15 minutes. The prepared 5-fold hemolysate is ready for use after being observed to be clear and transparent under light.

2、Operating Table:

	Blank	Contrast	Sample
Sample to assay (ml)			0.1
Distilled water (ml)	0.35		
8 mol/ml acetylcholine working solution (ml)		0.25	0.25
Reagent 1 (ml)	0.5	0.5	0.5
Mix sufficiently, place in 37℃ water bath for 20 minutes			
Reagent 3 working solution (ml)	1.0	1.0	1.0
Reagent 4 (ml)	0.5	0.5	0.5
Reagent 5 (ml)	0.25	0.25	0.25
Reagent 6 (ml)	0.5	0.5	0.5
Sample to assay (ml)		0.1	
Mix well, centrifuge at 3500 rpm for 10 minutes. Take the supernatant. Measure the absorbance A of each tube at 520 nm with a 1 cm path length. Zero the blank tube.			

3. **Calculation and Examples:**

Definition: Each milliliter of whole blood was incubated at 37°C for 20 minutes with the substrate, and 1 mole of acetylcholine was decomposed to 1 unit of activity.

Calculation Formula:

$$\textbf{Whole Blood CHE Activity(U/ml)}=\frac{A_{contrast}-A_{sample}}{A_{contrast}}\times C_{standard}\times V_{standard}\times\frac{1}{V_{sample}}\times N$$

$C_{standard}$: Acetylcholine concentration: 8 mol/mL;
$V_{standard}$: Acetylcholine Volume: 0.25 mL
V_{sample}: Tissue Sample Volume: 0.1 mL
N： Tissue Sample: Pre-test Dilution Factor

Example: Take fresh duck whole blood and follow the operation steps. The OD value of the

control tube was measured to be 0.531, and the OD value of the test tube was 0.435. The calculation results are as follows:

Whole Blood CHE Activity(U/ml)=

$$\frac{0.531-0.435}{0.531}\times 8\times 0.25\times \frac{1}{0.1}\times 5=18.08\ U/ml$$