



ISO 13485:2016 ISO 9001:2015

Ver.260501

## **Butyrylcholinesterase Activity Assay Kit**

BC5970-01 (50 Tests/24 Samples)

**FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS**

## Product Description

Butyrylcholinesterase (BchE), also known as plasma cholinesterase, pseudocholinesterase, is a serine hydrolyze that is synthesized by the liver and enters the blood and is present in almost all animal tissues. BchE is structurally similar to acetylcholinesterase (AChE), but with different substrate specificity and inhibitor sensitivity. Compared with AChE, BchE can effectively hydrolyze larger choline esters, such as butyrylcholine and benzoylcholine, and can remove the toxic effect of nerve agents such as organophosphorus pesticides and carbamate pesticides. Studies have shown that BchE can be an important target for the treatment of Alzheimer's disease.

BchE catalyzed the hydrolysis of butyrylcholine to choline, and the reaction of choline with disulfide p-nitrobenzoic acid (DTNB) to 5-merhydrl-nitrobenzoic acid (TNB). TNB has an absorption peak at 412nm. BchE activity was calculated by measuring the absorbance increase rate at 412nm.



## Kit components

Reagent	Volume	Storage
Extract solution	30mL	2-8°C
Reagent I	65mL	2-8°C
Reagent II	Powder	2-8°C
Reagent III	6mL	2-8°C
Reagent IV	Powder	2-8°C

## Reagent Preparation

### Reagent II

Add 9.7mL distilled water and gently mix to dissolve the contents. Unused reagents can be stored in aliquots at -20°C for 4 weeks.

### Reagent IV

Add 22mL Reagent I to the powder and gently mix to dissolve the contents. The solution is stable for 2 weeks at 2-8°C and for 2 months at -20°C. Prepare aliquots before freezing to avoid multiple freeze-thaw cycles.

## Reagents and Equipment Required but Not Provided

Spectrophotometer, low temperature centrifuge, analytical balance, water bath/constant temperature incubator, 1mL glass cuvette, adjustable pipette, mortar/homogenizer/cell ultrasonic crusher, ice, 1.5mL microcentrifuge tube and distilled water.

## Protocol

### I. Sample Preparation

- Tissues:** According to the tissues mass (g): Extract solution volume (mL) is the ratio of 1:5~10 (suggest that take 0.1g tissues and add 1mL extract solution) on the ice bath to homogenate. Centrifuge at  $8000 \times g$ , 4°C for 10 minutes, take the supernatant for test.
- Bacteria or cells:** According to the number of cells ( $10^4$ ), the proportion of Extract solution volume (mL) is 500~1000=1:1 (Suggest that add 1mL of Extract solution to 5 million cells). Ultrasonic breaking (power 300W, ultrasonic 3 seconds, interval 7 seconds, total time 3 minutes) on ice; Then Centrifuge at  $8000 \times g$ , 4°C for 10 minutes, take the supernatant on ice for test.
- Serum and other liquids:** Direct determination.

## II. Determination procedure

1. Preheat the spectrophotometer for 30 minutes, adjust the wavelength to 412nm and set the counter to zero with distilled water.
2. Operation table:

Reagent (μL)	Test tube (T)	Control tube (C)
Sample	30	30
Reagent II	100	-
Incubate at 37°C for 5 minutes		
Reagent IV	100	100
Reagent II	-	100
Reagent III	100	100
Mix thoroughly, centrifuge at 12000 rpm for 5 minutes. Pipette 50μL of the supernatant into the 1.5mL microcentrifuge tube and add it separately		
Reagent I	950	950
Mix thoroughly, stay for 2 minutes, determine the absorbance at 412nm, record absorbance of T and C as A <sub>T</sub> and A <sub>C</sub> respectively. Calculate ΔA=A <sub>T</sub> -A <sub>C</sub> .		

## Calculations

### 1. Tissue

#### a) Protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the generation of 1nmol TNB in the reaction system per minute for every mg protein.

$$\text{BchE activity (U/mg prot)} = [\Delta A \div (\epsilon \times d) \times V_C \times 10^9] \div (C_{pr} \times V_S \times V_{SU} \div V_{EN}) \div T \\ = 2255 \times \Delta A \div C_{pr}$$

#### b) Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the generation of 1nmol TNB in the reaction system per minute for every g sample.

$$\text{BchE activity (U/g fresh weight)} = [\Delta A \div (\epsilon \times d) \times V_C \times 10^9] \div (W \times V_S \div V_{TS} \times V_{SU} \div V_{EN}) \div T \\ = 2255 \times \Delta A \div W$$

### 2. Bacteria and cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the generation of 1nmol TNB in the reaction system per minute for every 10<sup>4</sup> cells.

$$\text{BchE activity (U/10}^4\text{cell)} = [\Delta A \div (\epsilon \times d) \times V_C \times 10^9] \div (N \times V_S \div V_{TS} \times V_{SU} \div V_{EN}) \div T \\ = 2255 \times \Delta A \div N$$

### 3. Serum

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1nmol TNB in the reaction system per minute for every mL serum.

$$\text{BchE activity (U/mL)} = [\Delta A \div (\epsilon \times d) \times V_C \times 10^9] \div (V_S \times V_{SU} \div V_{EN}) \div T \\ = 2255 \times \Delta A$$

$\epsilon$  : The molar extinction coefficient of TNB is  $13.6 \times 10^3 \text{ L/mol/cm}$   
 $d$  : Light path of cuvette, 1 cm  
 $V_C$  : Total volume of color reaction system (L),  $1 \text{ mL} = 0.001 \text{ L}$   
 $10^9$  : Unit conversion factor,  $1 \text{ mol} = 1 \times 10^9 \text{ nmol}$   
 $V_{EN}$  : Total volume of enzymatic reaction, 0.23 mL  
 $V_{SU}$  : Supernatant volume, 0.05 mL  
 $V_{TS}$  : Extraction volume, 1 mL  
 $C_{pr}$  : Protein concentration, mg/mL  
 $W$  : Sample weight, g  
 $V_S$  : Sample volume, 0.03 mL  
 $T$  : Reaction time, 5 minutes  
 $N$  : The number of cells extracted,  $10^4$

## Note

1. During the determination process, the sample should be placed on ice to avoid denaturation and inactivation.
2. When the absorbance is over than 1, it is recommended to dilute the sample for determination. Final value has to be multiplied by the dilution factor.
3. Each sample requires a Test tube and a Control tube.