

# Butyrylcholinesterase Activity Assay

**Detection Equipment:** Spectrophotometer

**Catalog Number:** BC5970

**Size:** 50T/48S

**Components:** Please carefully check the volume of the reagent and the volume in the bottle before use. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation condition
Reagent I	Liquid 100 mL×1	2-8°C storage
Reagent II	Powder ×1	-20°C storage
Reagent III	Liquid 30 mL×1	2-8°C storage

Solution reparation :

1. Reagent II: Add 30mL Reagent I before clinical use, dissolve fully, storage at -20°C for 4 weeks, avoid repeated freezing and thawing.

## Description:

Butyrylcholinesterase (BchE, EC3.1.1.8), also known as plasma cholinesterase, pseudocholinesterase, is a serine hydrolase 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-merhydryl-nitrobenzoic acid (TNB). TNB has an absorption peak at 412nm. BchE activity was calculated by measuring the absorbance increase rate at 412nm.



**Note:** Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment. If the absorbance value of the sample is not within the measurement range, it is recommended to dilute or increase the sample size for detection.

## 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 and distilled water.

## Protocol:

### I. Sample processing (The sample size to be tested can be appropriately adjusted, and the specific proportion can be referred to the literature)

**1. Tissue sample:** Add Reagent I according to the ratio of tissue mass (g) : Reagent I volume (mL) = 1:5 ~10 (it is recommended to weigh 0.1g sample and add 1.0mL Reagent I), after ice bath homogenization, centrifuge at 4°C, 12000rpm for 10min, discard precipitation, take supernatant and put it on ice to be measured.

**2. Serum/plasma and other liquid samples:** Direct measurement. If there is turbidity, centrifuge, take the supernatant and put it on the ice to be measured.

**3. Bacteria, cells:** According to the number of cells  $10^4$ : Reagent I volume (mL) 500~1000:1 ratio (it is recommended to add 1 mL Reagent I to 5 million cells), ice bath ultrasonic crushing cells (power 300w, ultrasonic 3s, 7s interval, total time 3 min), centrifuge at 4°C, 12000rpm for 10min, discard precipitation, take superserum placed on ice to be measured.

### II. Measurement Steps

1. Spectrophotometer for more than 30min, adjust the wavelength to 412nm, and zero the distilled water.

2. Operation table: (Add the following reagents in 1mL glass cuvette)

Reagent name (μL)	Test tube	Blank tube
Sample	50	-
Distilled water	-	50
Reagent II	500	500
Reagent III	500	500

Immediately and thoroughly mixed, the absorption value A1 at 10s was measured at 412nm, quickly placed in a 37°C water bath or constant temperature incubator for 5min, and quickly wiped dry to determine the absorption value A2 at 5min10s. Calculate  $\Delta A_{\text{text}} = A_{\text{text}2} - A_{\text{text}1}$ ,  $\Delta A_{\text{blank}} = A_{\text{blank}2} - A_{\text{blank}1}$ ,  $\Delta A = \Delta A_{\text{text}} - \Delta A_{\text{blank}}$ . The blank tube only needs to be measured 1-2 times.

### III. Calculations

#### 1. Calculated by protein concentration

Activity unit definition: One unit of activity was defined as 1nmol TNB per minute catalyzed per mg of protein.

$$\text{BchE activity (U/mg prot)} = [\Delta A \div (\epsilon \times d) \times V_{\text{total reaction}} \times 10^9] \div (C_{\text{pr}} \times V_{\text{sample}}) \div T \times F = 308.8 \times \Delta A \div C_{\text{pr}} \times F。$$

#### 2. Calculated by sample quality

Activity unit definition: One unit of activity was defined as 1nmol TNB per minute catalyzed by g of tissue.

$$\text{BchE activity (U/g mass)} = [\Delta A \div (\epsilon \times d) \times V_{\text{total reaction}} \times 10^9] \div (W \times V_{\text{sample}} \div V_{\text{total sample}}) \div T \times F = 308.8 \times \Delta A \div W \times F。$$

#### 3. Calculated by the volume of serum/plasma and other liquids

Activity unit definition: One unit of activity was defined as 1nmol TNB per minute catalyzed

per mL of serum/plasma.

$$\text{BchE activity (U/mL)} = [\Delta A \div (\epsilon \times d) \times V_{\text{total reaction}} \times 10^9] \div V_{\text{sample}} \div T \times F = 308.8 \times \Delta A \times F。$$

#### 4. Calculated by number of bacteria/cells

Activity unit definition: Activity unit was defined as 1nmol TNB per 10 000 cells per minute catalyzed production.

$$\text{BchE activity (U/10}^4 \text{ cell)} = [\Delta A \div (\epsilon \times d) \times V_{\text{total reaction}} \times 10^9] \div (N \times V_{\text{sample}} \div V_{\text{total sample}}) \div T \times F = 308.8 \times \Delta A \div N \times F。$$

$\epsilon$  : TNB molar extinction coefficient,  $13.6 \times 10^3 \text{ L/mol/cm}$ ;

d: light diameter of cuvette, 1cm;

$V_{\text{total reaction}}$ : total volume of reaction system,  $1.05 \text{ mL} = 1.05 \times 10^{-3} \text{ L}$ ;

$10^9$ : unit conversion coefficient,  $1 \text{ mol} = 1 \times 10^9 \text{ nmol}$ ;

$V_{\text{sample}}$ : sample volume added to the reaction system, 0.05mL;

$V_{\text{total sample}}$ : add one volume of reagent, 1mL;

Cpr: protein concentration, mg/mL;

W: sample quality, g;

T: reaction time, 5min;

F: sample dilution;

N: number of bacteria/cell, in tens of thousands.

#### Note:

- In order to ensure the accuracy of the results, please strictly control the reaction time. It is recommended that two people conduct the experiment, one person adds the sample, and one person timing.
- If the  $\Delta A_{\text{text}}$  is close to the  $\Delta A_{\text{blank}}$ , the sample size can be increased before the measurement; If the  $A_{2\text{ assay}}$  is greater than 1 or the  $\Delta A_{\text{text}}$  is greater than 0.7, it is recommended that the sample supernatant be appropriately diluted with reagent one before the assay is performed. Note that the calculation formula is modified synchronously.

#### Experimental example:

- Take 0.1018g of rat liver sample, add 1 ml of Reagent I for ice bath homogenization, after centrifugation, the supernatant was diluted 4 times with Reagent I, according to the determination steps, and measured by 1 ml glass cuvette calculation:  $\Delta A_{\text{text}} = A_{\text{text}2} - A_{\text{text}1} = 0.6807 - 0.3913 = 0.2894$ ,  $\Delta A_{\text{blank}} = A_{\text{blank}2} - A_{\text{blank}1} = 0.3552 - 0.2931 = 0.0621$ ,  $\Delta A = \Delta A_{\text{text}} - \Delta A_{\text{blank}} = 0.2273$ , calculated by sample quality:

$$\text{BchE activity (U/g mass)} = [\Delta A \div (\epsilon \times d) \times V_{\text{total reaction}} \times 10^9] \div (W \times V_{\text{sample}} \div V_{\text{total sample}}) \div T \times F = 2757.966 \text{ U/g 质量。}$$

- Take the horse serum sample, dilute 64 times with the reagent, operate according to the determination steps, and calculate the results by measuring with 1mL glass cuvette:  $\Delta A_{\text{text}} = A_{\text{text}2} - A_{\text{text}1} = 0.5100 - 0.3436 = 0.1664$ ,  $\Delta A_{\text{blank}} = A_{\text{blank}2} - A_{\text{blank}1} = 0.3552 - 0.2931 = 0.0621$ ,  $\Delta A = \Delta A_{\text{text}} - \Delta A_{\text{blank}} = 0.1043$ , calculated by liquid volume:

$$\text{BchE activity (U/mL)} = [\Delta A \div (\epsilon \times d) \times V_{\text{total reaction}} \times 10^9] \div V_{\text{sample}} \div T \times F = 2061.302 \text{ U/mL。}$$

## References:

- [1] Ellman GL, Courtney KD, Andres V Jr. et al. A new and rapid colorimetric determination of acetylcholinesterase activity [J]. Biochemical Pharmacology, 1961, 7(2): 88-95.
- [2] Yücel YY, Tacal O, Ozer I. Comparative effects of cationic triarylmethane, phenoxazine and phenothiazine dyes on horse serum butyrylcholinesterase [J]. Archives of Biochemistry and Biophysics, 2008, 478(2): 201-205.
- [3] Jońca J, Żuk M, Wasąg B. et al. New Insights into Butyrylcholinesterase Activity Assay: Serum Dilution Factor as a Crucial Parameter [J]. PLoS ONE, 2015, 10(10).

## Related products:

- BC0840/BC0845 羧酸酯酶 (CarE) 活性检测试剂盒
- BC2020/BC2025 乙酰胆碱酯酶 (AChE) 活性检测试剂盒
- BC2130/BC2135 组织及血液酸性磷酸酶 (ACP) 活性检测试剂盒
- BC2140/BC2145 组织及血液碱性磷酸酶 (AKP/ALP) 活性检测试剂盒