Ver.260101

Butyrylcholinesterase Activity Assay Kit

BC5970 (50Tests/48Samples)

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

I. Product Description

Butyrylcholinesterase, 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.

$$\text{Butyrylcholine} \xrightarrow{\text{BchE}} \text{Choline} \xrightarrow{\text{DTNB}} \text{TNB (412nm)}$$

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.

II. Reagent Composition & Preparation

Reagent	Volume	Storage
Reagent I	100mL	2-8℃
Reagent II	Powder×1	-20℃
Add 30mL Reagent I before clinical use, dissolve fully, storage at -20℃for 4weeks, avoid repeated freezing and thawing		
Reagent III	30mL	2-8℃

III. 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.

IV. Protocol

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

a) 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℃, 12000rpm for 10minutes, discard precipitation, take supernatant and put it on ice to be measured.

b) 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.

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

II. Measurement Steps

a) Spectrophotometer for more than 30minutes, adjust the wavelength to 412nm, and zero the distilled water.

b) 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℃ water bath or constant temperature incubator for 5minutes, and quickly wiped dry to determine the absorption value A2at 5minutes10s. Calculate $\Delta A_T = A_{T2} - A_{T2}$, $\Delta A_B = A_{B2} - A_{B1}$, $\Delta A = \Delta A_T - \Delta A_B$. The blank tube only needs to be measured 1-2 times		

III. Calculation

a) Calculated by protein concentration

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

$$\textbf{BchE activity (U/mg prot)} = [\Delta A \div (\varepsilon \times d) \times V_T \times 10^9] \div (Cpr \times V_S) \div T \times F$$
$$= 308.8 \times \Delta A \div Cpr \times F$$

b) Calculated by sample quality

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

$$\textbf{BchE activity (U/g mass)} = [\Delta A \div (\varepsilon \times d) \times V_T \times 10^9] \div (W \times V_S \div V_T) \div T \times F$$
$$= 308.8 \times \Delta A \div W \times F$$

c) 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.

$$\textbf{BchE activity (U/mL)} = [\Delta A \div (\varepsilon \times d) \times V_T \times 10^9] \div V_S \div T \times F$$
$$= 308.8 \times \Delta A \times F$$

d) Calculated by number of bacteria/cells

Activity unit definition: Activity unit was defined as 1nmol TNB per 10000 cells per minute catalyzed production.

$$\textbf{BchE activity (U/10}^4\textbf{ cell)} = [\Delta A \div (\varepsilon \times d) \times V_T \times 10^9] \div (N \times V_S \div V_T) \div T \times F$$
$$= 308.8 \times \Delta A \div N \times F$$

ε: TNB molar extinction coefficient, 13.6×10^{3}L/mol/cm;
d: light diameter of cuvette, 1cm;
V_T: Total volume of reaction system, 1.05mL=1.05×10^{-3} L;
10^9: unit conversion coefficient, 1mol=1×10^{9}nmol;
V_S: sample volume added to the reaction system, 0.05mL;
V_T: add one volume of reagent, 1mL;
Cpr: protein concentration, mg/mL;
W: sample quality, g;
T: reaction time, 5minutes;
F: sample dilution;
N: number of bacteria/cell, in tens of thousands.

Note:

1. 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.
2. If the ΔA_T is close to the ΔA_B, the sample size can be increased before the measurement; If the A2 >1 or the $\Delta A_T > 0.7$, 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.