Butyrylcholinesterase Activity Assay Kit

Detection Equipment: Spectrophotometer/Microplate Reader

Catalog Number: BC5975

Size: 100T/96S

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 125 mL×1	2-8℃ storage
Reagent II	Powder ×1	-20℃ storage
Reagent III	Liquid 12 mL×1	2-8℃ storage

Solution reparation :

1. Reagent II: Add 12mL Reagent I before clinical use, dissolve fully, storage at -20℃ 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/Microplate Reader, low temperature centrifuge, analytical balance, water bath/constant temperature incubator, micro glass cuvette/96 well flat-bottom plate, 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℃, 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℃, 12000rpm for 10min, discard precipitation, take superserum placed on ice to be measured.

II. Measurement Steps

1. Spectrophotometer/Microplate Reader for more than 30min, adjust the wavelength to 412nm, and zero the distilled water.
2. Operation table: (Add the following reagents in micro glass cuvette/96 well flat-bottom plate)

Reagent name（μL）	Test tube	Blank tube
Sample	10	-
Distilled water	-	10
Reagent II	100	100
Reagent III	100	100
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 5min(The enzyme marker has the temperature control function to adjust the temperature to 37℃), and quickly wiped dry to determine the absorption value A2 at 5min10s. Calculate $\Delta A_{text}=A_{text2}-A_{text1}$, $\Delta A_{blank}=A_{blank2}-A_{blank1}$, $\Delta A=\Delta A_{text}-\Delta A_{blank}$. The blank tube only needs to be measured 1-2 times.		

III. Calculations

1. With micro glass cuvette determination:

1) Calculated by protein concentration

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

BchE activity(U/mg prot)=[ΔA÷（ε×d）×$V_{total\ reaction}$×10^9] ÷（Cpr×V_{sample}）÷T×F=308.8×ΔA÷Cpr×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.

BchE activity(U/g mass)=[ΔA÷（ε×d）×$V_{total\ reaction}$×10^9]÷（W×V_{sample}÷$V_{total\ sample}$）÷T×F = 308.8 × ΔA ÷ W×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.

BchE activity(U/mL)=[ΔA÷（ε×d）×$V_{total\ reaction}$×10^9]÷V_{sample}÷T×F=308.8×ΔA×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.

BchE activity(U/10^4 cell)=[ΔA÷（ε×d）×$V_{total\ reaction}$×10^9]÷（N×V_{sample}÷$V_{total\ sample}$）÷T×F=308.8×ΔA÷N×F。

ε : TNB molar extinction coefficient, 13.6×10^3L/mol/cm;

d: light diameter of cuvette, 1cm;

$V_{total\ reaction}$: total volume of reaction system, 0.21mL=2.1×10^{-4}L;

10^9: unit conversion coefficient, 1mol=1×10^9nmol;

V_{sample}: sample volume added to the reaction system, 0.01mL;

$V_{total\ sample}$: 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.

2. With 96 well flat-bottom plate:

Change d-1cm in the above formula to d-0.6cm for calculation.

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_{text} is close to the ΔA_{blank}, the sample size can be increased before the measurement; If the A2 assay is greater than 1 or the ΔA_{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:

1. Take 0.1018g of rat liver sample, add 1 ml of Reagent I for ice bath homogenization, after centrifugation, the supernatant was diluted 2 times with Reagent I, according to the determination steps, and measured by 96 well flat-bottom plate calculation: $\Delta A_{text}=A_{text2}-A_{text1}$=0.636-0.417=0.219, $\Delta A_{blank}=A_{blank\ 2}-A_{blank\ 1}$= 0.188- 0.180= 0.008, $\Delta A=\Delta A_{text}-\Delta A_{blank}$ =0.211, calculated by sample quality(The optical diameter of 0.6cm is brought into the calculation):

   BchE activity（U/g mass）=[ΔA÷（ε×d）×$V_{total\ reaction}$×10^9]÷（W×V_{sample}÷$V_{total\ sample}$）÷T×F =2133.49 U/g 质量。

2. Take the horse serum sample, dilute 16 times with the reagent, operate according to the

determination steps, and calculate the results by measuring with 96 well flat-bottom plate: $\Delta A_{text}=A_{text2}-A_{text1}= 0.960- 0.299=0.661$, $\Delta A_{blank}=A_{blank\ 2}-A_{blank\ 1}= 0.188- 0.180=0.008$, $\Delta A=\Delta A_{text}-\Delta A_{blank}=0.653$, calculated by liquid volume(The optical diameter of 0.6cm is brought into the calculation):

BchE activity（U/mL）=[ΔA÷（ε×d）×$V_{total\ reaction}$×10^9]÷V_{sample}÷T×F =5377.237 U/mL。

References：

[1] Ellman GL, Courtney KD, Andres V Jr. et al. A new and rapid colorimetric deter 分钟 ation 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).

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