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Butyrylcholinesterase Inhibitor Activity Assay Kit

Detection Equipment: Spectrophotometer
Catalog Number: AK0905-50T-48S
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 us in time.

Reagent name	Size	Preservation condition
Extract	Liquid 60 mL×1	2-8℃ storage
Reagent I	Powder ×2	-20℃ storage
Reagent II	Liquid 40 mL×1	2-8℃ storage
Reagent III	Liquid 35 mL×1	2-8℃ storage
Reagent IV	Powder ×1	-20℃ storage
Reagent V	Liquid 1 mL×1	-20℃ storage

Solution reparation :

1. Reagent I: Before use, take 1 Reagent I, add 1.8mL distilled water, fully dissolve, and storage the reagent for 4 weeks after subpacking at -20℃ to avoid repeated freeze-thaw (The reagent is a freeze-dried reagent, there may be a large difference or even a small amount of macroscopic observation between different bottles, this phenomenon does not affect the use, the actual quality is the same).
2. Reagent IV: Add 17mL Reagent II before clinical use, fully dissolve, and the reagent can be storage for 4 weeks at -20℃ to avoid repeated freezing and thawing.
3. Reagent V:10mmol/L Livansmin solution. Before clinical use, 15μL 10mmol/L Livansmin solution was taken, and 985μL distilled water was added to prepare 0.15mmol/L Livansmin solution. (This reagent was used for positive tube experiment, and it was selected.)

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. Compared with acetylcholinesterase (AchE), BchE can effectively hydrolyze larger choline esters, such as butyrylcholine and benzoylcholine, and can remove the toxic effects of nerve agents such as organophosphorus pesticides and carbamate pesticides. Studies have shown that BchE can be used as an important target for the treatment of Alzheimer's disease, and BchE inhibitors are used to improve memory loss and cognitive dysfunction in Alzheimer's patients.

BchE catalyzes the hydrolysis of butyrylcholine to choline, and the reaction of choline with dithio-nitrobenzoic acid (DTNB) to produce 5-merhydryl-nitrobenzoic acid (TNB). BchE inhibitors reduce the hydrolysis of butyrylcholine by inhibiting BchE activity. TNB has an absorption peak at 412nm, and

the BchE inhibitor activity can be calculated by measuring the change of absorbance at 412nm.

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

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, balance, oven, pulverizer/mortar/homogenizer, 30-50 mesh screen, ultrasonic cleaning machine, centrifuge, water bath/constant temperature incubator, 1mL glass cuvette, adjustable pipette, 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: The sample was dried to constant weight, crushed, after 30-50 mesh sieve, weighed about 0.1g, added 1mL of extraction solution, and extracted by ultrasonic extraction method, ultrasonic power 300W, 60℃, extraction for 30min. 12000rpm, 25℃, centrifuge for 10min, take the supernatant, and volume with the extraction solution to 1mL, to be measured.

2. Powder sample: Take an appropriate amount of sample, add an appropriate amount of Extract , and prepare a solution with appropriate concentration to be measured.

Note: If the powder sample is insoluble in Extract, it can be dissolved with a suitable solvent, prepared into a solution of 100× or greater concentration, and then diluted to 1× concentration with the extraction solution.

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）	Blank tube 1	Blank tube 2	Test tube	Positive tube (optional)
Reagent I	50	-	50	50
Reagent II	250	300	250	250
Extract	50	50	-	-
Sample	-	-	50	-
Reagent V	-	-	-	50
Mix well and incubate at 25℃ for 10min in dark				
Reagent III	500	500	500	500
Reagent IV	250	250	250	250
Thoroughly mixed, the absorbance value A at 10s was measured at 412nm, and the absorbance value A 'at 5min10s was measured accurately at 37℃ for 5min, and was recorded as A blank 1, $A_{blank\ 2}$, A_{test}, $A_{positive}$ and $A'_{blank\ 1}$, $A'_{blank\ 2}$, A'_{test}, $A'_{positive}$, respectively. Calculate $\Delta A_{blank} = (A'_{blank\ 1} - A_{blank\ 1}) - (A'_{blank\ 2} - A_{blank\ 2})$, $\Delta A_{test} = (A'_{test} - A_{test}) - (A'_{blank\ 2} - A_{blank\ 2})$, $\Delta A_{positive} = (A'_{positive} - A_{positive}) - (A'_{blank\ 2} - A_{blank\ 2})$. Blank tubes 1 and 2 only need to be done 1-2 times.				

III. Calculations

1. Calculation of inhibition rate

BchE inhibitor inhibition rate (%) = (ΔA_{blank} -ΔA_{test}) ÷ΔA_{blank} ×100%.

2.IC_{50} calculation

IC_{50}, the semi-inhibitory concentration of the inhibitor. For the samples that are determined to have inhibition on BchE, appropriate concentration gradients can be formulated, and the sample concentration is taken as the horizontal coordinate and the inhibition rate as the vertical coordinate as the inhibition curve, so as to calculate the sample concentration when the inhibition rate is 50%, that is, IC_{50}.

Note:

1. In order to ensure the accuracy and stability of the experimental results, please strictly control the reaction time and operation time.
2. When the sample absorbance A'_{test} is greater than 1.5 or the ΔA_{test} is close to ΔA_{blank}, it is recommended to increase the proportion of tissue samples in the sample processing step or prepare the powder sample into a higher concentration solution before the determination. When the ΔA_{test} is less than 0.01, the sample can be diluted with the extraction solution and then determined.
3. If it is used to compare the degree of inhibition of BchE by different reagents, extracts, drugs or tissues, the reagent, extract, drug or tissue homogenate must be prepared to the same concentration for comparison.

Experimental example:

1. Take 0.1005g pomelo peel sample (dried), add 1mL Extract solution for ultrasonic extraction, centrifuge and take supernatant, according to the measurement procedure, and use 1mL glass cuvette to measure and calculate: $\Delta A_{blank} = (A'_{blank\ 1} - A_{blank\ 1}) - (A'_{blank\ 2} - A_{blank\ 2}) = (1.372-0.205) - (0.166-0.112) = 1.113$, $\Delta A_{test} = (A'_{test} - A_{test}) - (A'_{blank\ 2} - A_{blank\ 2}) = (1.117-0.485) - (0.166-0.112) = 0.578$, calculated inhibition rate:
   BchE inhibitor inhibition rate (%) = (1.113-0.578) ÷1.113×100%=48.068%.
2. Take 0.1014g persimmon peel sample (dried), add 1mL Extract solution for ultrasonic extraction, centrifuge and obtain supernant, dilute 16 times according to the measurement procedure, and use 1mL glass cuvette to measure and calculate: $\Delta A_{blank} = (A'_{blank\ 1} - A_{blank\ 1}) - (A'_{blank\ 2} - A_{blank\ 2}) = (1.372-0.205) - (0.166-0.112) = 1.113$, $\Delta A_{test} = (A'_{test} - A_{test}) - (A'_{blank\ 2} - A_{blank\ 2}) = (0.706-0.149) - (0.166-0.112) = 0.503$, the inhibition rate is calculated as follows:
   BchE inhibitor inhibition rate (%) = (1.113-0.503) ÷1.113×100%=54.807%.
3. Take 50μL 0.15mmol/L Livansmin solution, follow the measurement steps, and use 1mL glass cuvette to measure and calculate: $\Delta A_{blank} = (A'_{blank\ 1} - A_{blank\ 1}) - (A'_{blank\ 2} - A_{blank\ 2}) = (1.372-0.205) - (0.166-0.112) = 1.113$, $\Delta A_{test} = (A'_{test} - A_{test}) - (A'_{blank\ 2} - A_{blank\ 2}) = (0.838-0.171) - (0.166-0.112) = 0.613$, the inhibition rate is calculated as follows:
   BchE inhibitor inhibition rate (%) = (1.113-0.613) ÷1.113×100%=44.924%.

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] Noor Atatreh, Sara Al Rawashdah, Shaikha S Al Neyadi. et al. Discovery of new butyrylcholinesterase inhibitors via structure-based virtual screening [J]. Journal of Enzyme Inhibition and Medicinal Chemistry, 2019, 34(1): 1373-1379.

[3] Li Q, Chen Y, Xing S. et al. Highly Potent and Selective Butyrylcholinesterase Inhibitors for Cognitive Improvement and Neuroprotection [J]. Journal of Medicinal Chemistry, 2021, 64(10): 6856-6876.