（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-F076

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=325 nm/393 nm)

Detection range：0.003-4.75 U/L

Elabscience®β-Secretase 1 (BACE 1)

Activity Fluorometric Assay Kit

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623

Tell: 1-832-243-6086

Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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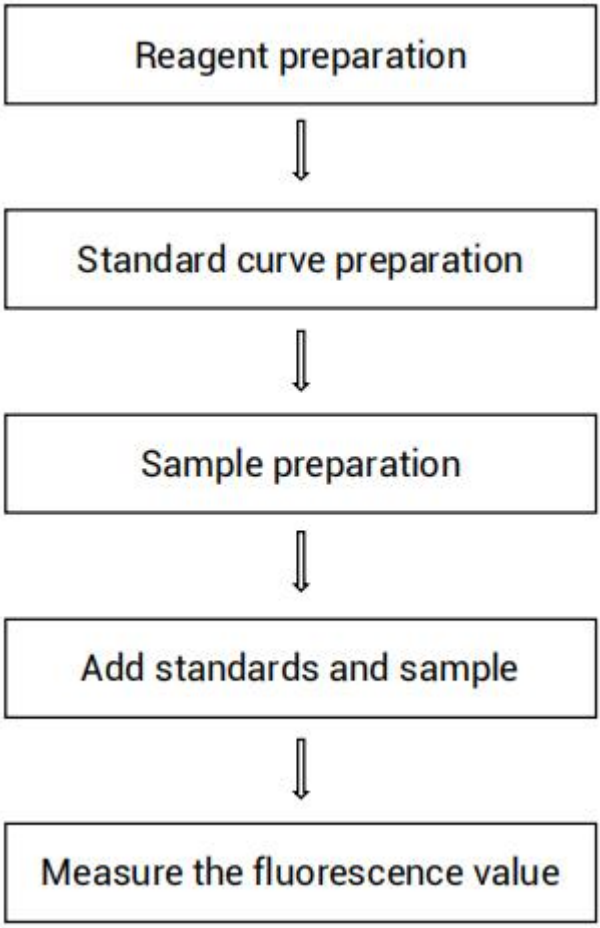
Assay summary



Intended use

This kit can be used to measure β-Secretase 1(BACE 1) activity in serum, plasma, tissue and cell samples.

Detection principle

β-Secretase 1 is an aspartic protease, the full name is β-site amyloid precursor protein cleavage enzyme 1(BACE 1). BACE 1 cleaves amyloid precursor protein (APP) to produce amyloid-β peptide (Aβ) of 40 or 42 amino acids. The deposition of Aβ in plaques and vessel walls in the brains of patients with Alzheimer's disease is widely believed to be the major contributor to the disease.

The detection principle of this kit is fluorescence resonance energy transfer (FRET) method. A fluorescence donor group and a quenching group are divided at both ends of the substrate. The fluorescence energy is transferred from the fluorescence donor to the quenching group, resulting in the attenuation of the fluorescence intensity of the fluorescence donor molecule itself. When the substrate is cleaved by BACE 1, the quenching group of the substrate and the fluorescent donor are separated and the fluorescent donor is no longer quenched, the fluorescence of the fluorescent donor can be detected, so that the enzymatic activity of BACE 1 can be calculated. The fluorescence produced by this kit had a maximum excitation wavelength of 325 nm and a maximum absorption wavelength of 393 nm.

Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Storage
Reagent 1	Buffer	15 mL × 1 vial	30 mL × 1 vial	-20°C, 12 months
Reagent 2	Extraction Solution	50 mL × 1 vial	50 mL × 2 vials	-20°C, 12 months shading light
Reagent 3	Substrate	0.25 mL × 1 vial	0.5 mL × 1 vial	-20°C, 12 months shading light
Reagent 4	Standard	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months shading light
	Black Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Fluorescence microplate reader (Ex/Em=325 nm/393 nm),

Incubator(37℃)

Reagents:

DMSO

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of standard working solution：
Dissolve one vial of standard with 860 μL of DMSO, mix well to dissolve. Store at -20°C for 7 days protected from light.

③ The preparation of 100 μmol/L standard solution：
Before testing, please prepare sufficient 100 μmol/L standard solution according to the test wells. For example, prepare 1000 μL of 100 μmol/L standard solution (mix well 10 μL of standard working solution and 990 μL of buffer). The 100 μmol/L standard solution should be prepared on spot and protected from light. The 100 μmol/L standard solution should be used up within 1 hour.

④ The preparation of working solution:
Before testing, please prepare sufficient working solution according to the test wells. For example, prepare 160 μL of working solution (mix well 5 μL of substrate and 155 μL of buffer). The working solution should be prepared on spot and protected from light.

⑤ The preparation of standard curve：
Always prepare a fresh set of standards. Discard working standard dilutions after use.
Dilute 100 μmol/L standard solution with double distilled water to a serial
concentration. The recommended dilution gradient is as follows: 0, 20, 30, 40, 60, 70, 80,100 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**20**	**30**	**40**	**60**	**70**	**80**	**100**
100 μmol/L standard (μL)	0	40	60	80	120	140	160	200
Double distilled water (μL)	200	160	140	120	80	60	40	0

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL extraction solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 200 μL extraction solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② **Dilution of sample**

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Mouse liver tissue homogenate	2-4
10% Mouse kidney tissue homogenate	2-4
10% Mouse heart tissue homogenate	1
10% Mouse brain tissue homogenate	1
Mouse plasma	1
1.4×10^6 Jurkat cell	1
1.4×10^6 A549 cell	1
1.6×10^6 293T cell	1
1.4×10^6 CHO cell	1

Note: The diluent is extraction solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard well: add 10 μL of standard with different concentrations into the wells.
Sample well: add 10 μL of sample into the wells.

② Add 120 μL of buffer into the standard wells. Add 120 μL of working solution into the sample wells.

③ Mix fully with microplate reader for 5 s. Measure the fluorescence intensity at the excitation wavelength of 325 nm and the emission wavelength of 393 nm, as F_1.

④ Incubate at 37°C for 20 min protected from light. Measure the fluorescence intensity at the excitation wavelength of 325 nm and the emission wavelength of 393 nm, as F_2 (Standard curves were fitted with F_2 values).

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean fluorescence value of the blank (Standard #①) from all standard readings. This is the absoluted fluorescence value.

3. Plot the standard curve by using absoluted fluorescence value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample:

Definition: The amount of substrate in 1 L serum or plasma sample that hydrolyze the substrate to produce 1 μmol product in 1 minute at 37°C is defined as 1 unit.

$$\text{BACE 1 activity (U/L)} = (\Delta F - b) \div a \div T \times f$$

2. Tissue and cell samples:

Definition: The amount of substrate in 1 g tissue or cell protein that hydrolyze the substrate to produce 1 μmol product in 1 minute at 37°C is defined as 1 unit.

$$\text{BACE 1 activity (U/gprot)} = (\Delta F - b) \div a \div T \times f \div C_{pr}$$

[Note]

ΔF: The change fluorescence value of sample, $F_2 - F_1$.

T: The incubation time, 20 min.

f: Dilution factor of sample before tested.

C_{pr}: Concentration of protein in tissue sample, gprot/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	1.00	1.50	2.50
%CV	2.2	2.5	3.2

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	1.00	1.50	2.50
%CV	4.7	9.2	10.4

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 100%.

	Standard 1	Standard 2	Standard 3
Expected Conc.(μmol/L)	25	50	75
Observed Conc.(μmol/L)	23.3	52.0	77.3
Recovery rate (%)	93	104	103

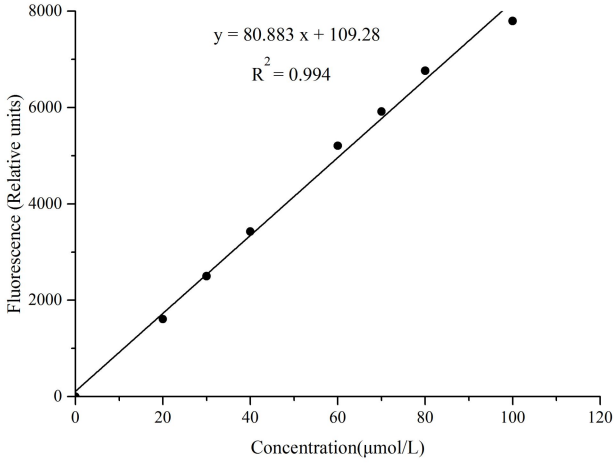
Sensitivity

The analytical sensitivity of the assay is 0.003 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

Concentration (μmol/L)	0	20	30	40	60	70	80	100
Fluorescence value	58	1689	2525	3483	5166	6077	6823	7825
	47	1633	2581	3474	5354	5866	6816	7878
Average fluorescence value	53	1661	2553	3478	5260	5971	6819	7852
Absoluted fluorescence value	0	1609	2500	3426	5208	5919	6767	7799



Appendix Π Example Analysis

Example analysis：

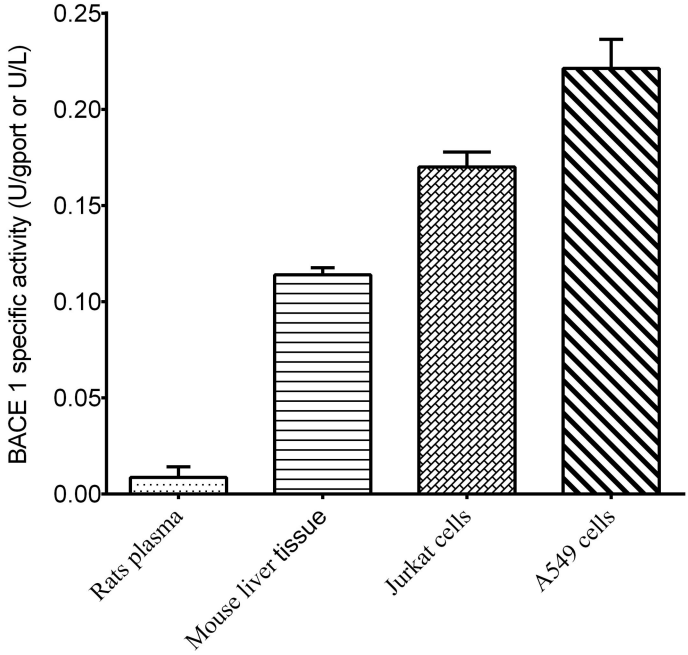
For 10% mouse liver tissue homogenate, dilute for 2 times, take 10 μL for detection and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 80.883 x + 109.28, the fluorescence value F_1 of the sample is 483, the fluorescence value F_2 of the sample is 1551, $\Delta F = F_2 - F_1 = 1551 - 483 = 1068$, the concentration of protein in sample is 10.67 gprot/L, and the calculation result is:

$$\text{BACE 1 activity (U/gprot)} = (1068 - 109.28) \div 80.883 \div 20 \times 2 \div 10.67$$

$$= 0.111 \text{ U /gprot}$$

Detect rats plasma, 10% mouse liver tissue homogenate (the concentration of protein is 10.67 gprot/L), 1.42×10^6 Jurkat cells (the concentration of protein is 0.71 gprot/L) and 1.48×10^6 A549 cells (the concentration of protein is 0.62 gprot/L)according to the protocol, the result is as follows:


BACE 1 specific activity (U/gport or U/L)
0.25
0.20
0.15
0.10
0.05
0.00
Rats plasma
Mouse liver tissue
Jurkat cells
A549 cells

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.