TECHNICAL MANUAL

β-Secretase 1 (BACE 1) Activity Fluorometric Assay Kit

- **SKU CODE:** MAES0319
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Incubate and measure fluorescence

2. Intended use

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

3. Detection principle

β-Secretase 1 is an aspartic protease, with the full name 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 the fluorescence resonance energy transfer (FRET) method. A fluorescence donor group and a quenching group are positioned at both ends of the substrate. The fluorescence energy is transferred from the fluorescence donor to the quenching group, resulting in 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 then be detected, allowing calculation of the enzymatic activity of BACE 1. The fluorescence produced by this kit has a maximum excitation wavelength of 325 nm and a maximum emission wavelength of 393 nm.

4. Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Storage
Reagent 1	Buffer	15 mL x 1 vial	30 mL x 1 vial	-20 °C, 12 months

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

5. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **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. **Preparation of 100 µmol/L standard solution:** Before testing, 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 fresh and protected from light. Use within 1 hour.
4. **Preparation of working solution:** Before testing, 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 fresh and protected from light.
5. **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 serial concentrations. The recommended dilution gradient is as follows: 0, 20, 30, 40, 60, 70, 80, 100 µmol/L.

6. Sample preparation

Serum and plasma: Detect directly. If not detected on the same day, the serum or plasma can be stored at -80 °C for one 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 °C.

4. Centrifuge at 10000 xg for 10 min at 4 °C to remove insoluble material. Collect supernatant and keep on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant.

Cell (adherent or suspension) samples:

1. Harvest the number of cells needed for each assay (initial recommendation $1x10^6$ cells).

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

3. Homogenize $1x10^6$ cells in 200 µL extraction solution with a dounce homogenizer at 4 °C.

4. Centrifuge at 10000 xg for 10 min at 4 °C to remove insoluble material. Collect supernatant and keep on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant.

7. Operating steps

1. Standard wells: Add 10 µL of standard with different concentrations into the wells. Sample wells: 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 thoroughly with microplate reader for 5 seconds. Measure the fluorescence intensity at excitation wavelength of 325 nm and emission wavelength of 393 nm, record as F1.

4. Incubate at 37 °C for 20 minutes protected from light. Measure the fluorescence intensity at excitation wavelength of 325 nm and emission wavelength of 393 nm, record as F2 (Standard curves are fitted with F2 values).

8. 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 absolute fluorescence value.

3. Plot the standard curve using absolute 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 hydrolyzes the substrate to produce 1 μmol product in 1 minute at 37 °C is defined as 1 unit.

BACE 1 activity (U/L) = (ΔF - b) ÷ a ÷ T × f

2. Tissue and cell samples:

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

BACE 1 activity (U/gprot) = (ΔF - b) ÷ a ÷ T × f ÷ C_{pr}

[Note]

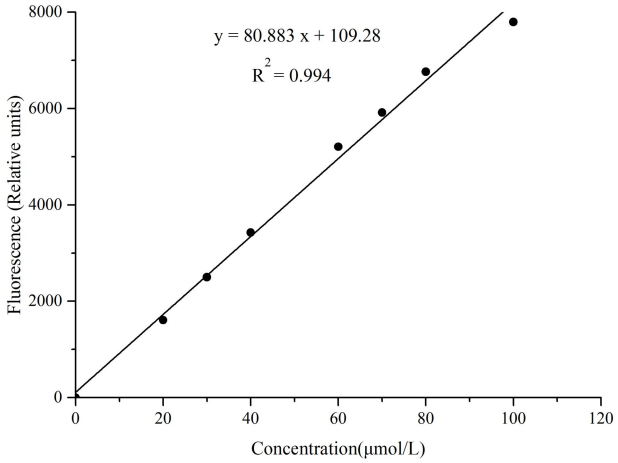
ΔF: The change in fluorescence value of sample, $F_2 – F_1$.

T: The incubation time, 20 min.

f: Dilution factor of sample before tested.

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

9. Appendix I Performance Characteristics



Intra-assay Precision

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

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

Recovery

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

Sensitivity

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

Standard curve data

Concentration (µmol/L)	Fluorescence value 1	Fluorescence value 2	Average fluorescence value	Absolute fluorescence value
0	58	47	53	0
20	1689	1633	1661	1609

Concentration (μmol/L)	Fluorescence value 1	Fluorescence value 2	Average fluorescence value	Absolute fluorescence value
30	2525	2581	2553	2500
40	3483	3474	3478	3426
60	5166	5354	5260	5208
70	6077	5866	5971	5919
80	6823	6816	6819	6767
100	7825	7878	7852	7799

10. Appendix II Example Analysis

Example analysis:

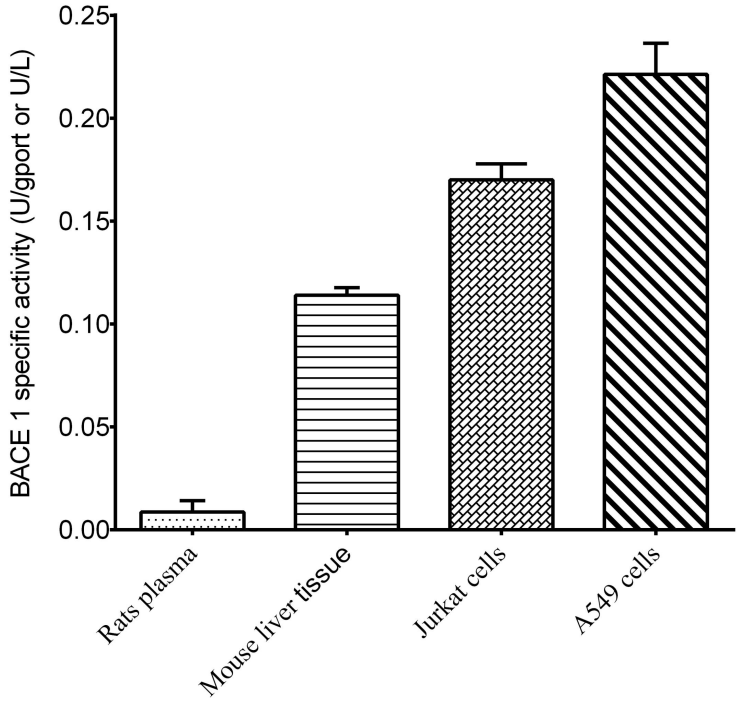
For 10% mouse liver tissue homogenate, dilute 2-fold, take 10 μL for detection and carry out the assay according to the operating steps. The results are as follows:

Standard curve: y = 80.883x + 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:

BACE 1 activity (U/gprot) = (1068 – 109.28) ÷ 80.883 ÷ 20 × 2 ÷ 10.67 = 0.111 U/gprot

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



11. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.