

Product Information

β -Secretase (BACE1) Activity Detection Kit (Fluorescent)

Catalog Number **CS0010**
Storage Temperature $-20\text{ }^{\circ}\text{C}$

TECHNICAL BULLETIN

Product Description

BACE1 (β -secretase or beta-site APP-cleaving enzyme) and γ -secretase are proteases that cleave the amyloid precursor protein (APP) to produce amyloid beta peptide ($A\beta$). The accumulation of $A\beta$ in the brain is believed to be a primary cause for the progression of Alzheimer's disease. Since there is no effective drug for the treatment of Alzheimer's disease, there is an intense interest in studying the inhibition of γ - and β -secretases for therapeutic intervention in Alzheimer patients.¹⁻³ Knockout studies show that BACE1 is critical for $A\beta$ generation. Transgenic mice lacking BACE1 do not produce $A\beta$, but show an otherwise normal phenotype with no detrimental effects on viability or morphology.⁴ This raises the possibility that therapeutic BACE1 inhibition could be accomplished without major toxicity.

BACE1 Activity Assay Kit is designed for BACE1 inhibitor screening. It provides all the reagents, including the BACE1 enzyme for use as a positive control, required for an efficient detection of BACE1 activity. The assay is based on a convenient method of fluorescence resonance energy transfer (FRET) in which the fluorescence signal enhancement is observed after the substrate is cleaved by BACE1.

Reagents

The kit contains sufficient reagents for 250 reactions in 96 well plate format.

Fluorescent Assay Buffer Catalog Number F8303	50 ml
Stop Solution Catalog Number S6318	15 ml
Assay Standard, 140 μ l Catalog Number A3103	1 vial

7-Methoxycoumarin-4-acetyl
[Asn⁶⁷⁰, Lue⁶⁷¹]-Amyloid β /A4 Protein
Precursor 770 Fragment 667-676-
(2,4 dinitrophenyl) Lys-Arg-Arg amide
trifluoroacetate salt (BACE1 substrate)
Catalog Number A1472

BACE1 100 μ l
 β -secretase activity, \sim 300 units
Unit definition: one unit will hydrolyze 1.0 picomole of
7-Methoxycoumarin-4-acetyl-[Asn⁶⁷⁰, Leu⁶⁷¹]-
Amyloid β /A4 Protein Precursor 770 Fragment 667-676
2,4-dinitrophenyl)Lys-Arg-Arg amide substrate per
minute at pH 4.5 at 25 $^{\circ}\text{C}$.
Catalog Number B9059

Equipment and Reagents Required but Not Provided

- Fluorometer
- 96 well plate for fluorescence assay
- Dimethyl Sulfoxide
(DMSO, Catalog Number D8418)
- [Asn⁶⁷⁰, Sta⁶⁷¹, Val⁶⁷²]-Amyloid β /A4 Precursor
Protein 770 Fragment 662-675 ammonium salt
(BACE1 inhibitor, optional - Catalog Number
A1847)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please refer to the Material Safety Data Sheet (MSDS) for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on dry ice and stored at $-20\text{ }^{\circ}\text{C}$. After first thaw, store the Fluorescent Assay Buffer and Stop Solution at 2–8 $^{\circ}\text{C}$. Avoid multiple freeze thaw cycles of the substrate, enzyme, and standard.

Preparation Instructions

Note: The volume of reagents detailed below is for assays performed in 96 well plates. For plates or wells of different sizes, adjust the amount of reagents required accordingly.

BACE1 Substrate Solution - Prepare a 1 mg/ml (500 μ M) stock solution by adding 0.5 ml of DMSO to the BACE1 substrate (Catalog Number A1472). Aliquot the BACE1 Substrate Solution and store at -20°C .

Just before beginning the assay, dilute an aliquot of the 500 μ M BACE1 Substrate Solution 10-fold, to 50 μ M, with Fluorescent Assay Buffer (Catalog Number F8303). Mix well.

BACE1 Enzyme Solution - Just before beginning the assay, dilute the BACE1 (Catalog Number B9059) 10-fold with Fluorescent Assay Buffer (Catalog Number F8303) to ~ 0.3 unit/ μ l. Mix well.

Assay Standard Solution - Just before beginning the assay, dilute the Assay Standard (Catalog Number A3103) 10-fold with Fluorescent Assay Buffer (Catalog Number F8303). Mix well. This will give a 100 μ M Assay Standard Solution.

Procedure

1. Set the fluorometer on well plate reader mode with excitation at 320 nm and emission at 405 nm.
2. Bring all components (except the BACE1 Enzyme Solution) to room temperature.
3. Add components to a fluorometer 96 well plate according to Table 1. Mix well by gentle pipetting. Add the BACE1 Enzyme Solution just before reading.

Table 1.

Reaction scheme

Reaction No.	1	2	3	4	5	6	7	8
Assay Description:	Negative Control: no enzyme	Positive Control: Supplied Enzyme activity	Inhibition	Test: Sample enzyme activity	Standard Curve			
Fluorescent Assay Buffer	80 μ l	78 μ l	78-X μ l	80-Y μ l	79 μ l	78 μ l	77 μ l	75 μ l
BACE1 Substrate Solution, 50 μ M	20 μ l	20 μ l	20 μ l	20 μ l	20 μ l	20 μ l	20 μ l	20 μ l
BACE1 Enzyme Solution, 0.3 units/ μ l	–	2 μ l	2 μ l	–	–	–	–	–
Inhibitor Solution			X μ l					
Sample enzyme				Y μ l				
Assay Standard Solution, 100 μ M	–	–	–	–	1 μ l (100 pmol)	2 μ l (200 pmol)	3 μ l (300 pmol)	5 μ l (500 pmol)
Total	100 μ l	100 μ l	100 μ l	100 μ l	100 μ l	100 μ l	100 μ l	100 μ l

Reaction 1: negative control (no enzyme) reaction and standard curve blank. The “blank” reaction tube reflects fluorescence due to substrate alone.

Reaction 2: supplied enzyme activity test - a positive control.

Reaction 3: enzyme inhibition test.

Reaction 4: sample enzyme activity test. **Notes:**

- For sample enzyme activity test use 20–30 μ l from a lysate of 5–7 mg/ml protein concentration.
- BACE1 activity may be affected by the extraction/formulation buffer used in enzyme preparation (see the Troubleshooting section).
- In order to verify the specificity of the activity measured, due to possible non-specific activity in tested lysates, it is highly recommended to add a test tube containing BACE1 specific inhibitor [Asn670, Sta671, Val672]-Amyloid β /A4 Precursor Protein 770 Fragment 662-675 (Catalog Number A1847). The inhibition profile of purified BACE1 activity by this inhibitor is described in Figure 2.

Reactions 5–8: standard curve

- Read the fluorescence immediately after adding the enzyme. This is “time zero” reading. The signal in the wells could increase between the addition of enzyme and this initial reading.
- Cover the plate with a Parafilm® and incubate at 37 °C for 2 hours.
- Read the signal at “time zero” plus 2 hours. The plate should be at room temperature before reading.
- Optional: After the readings are made, add 40 µl of Stop Solution. The addition of the Stop Solution will stabilize the signal for at least 24 hours.

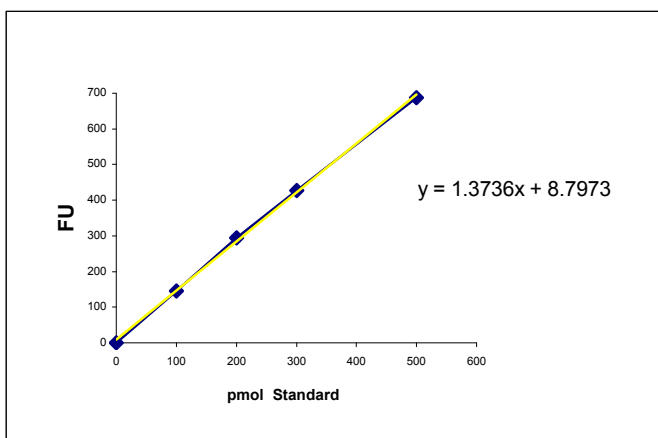
Calculations

The assay is designed so that the enzyme converts 5–20% of the substrate to a fluorescent product during 1–2 hours at 37 °C.

Determine Standard Curve:

- Subtract the fluorescence units (FU) of the blank value (reaction 1) from all signal readings (reactions 5–8) at 2 hours.
- Plot the fluorescence units against pmol present in each standard (see Figure 1).

Figure 1.
Typical Standard Curve



Standard curve obtained using Perkin Elmer luminescence spectrometer LS50B, excitation 320 nm (slit 12 nm) and emission 405 nm (slit 12 nm). The reaction scheme (tubes 1, 5–8) described in Table 1 was used.

Calculation of the percentage of substrate cleavage

The percentage of substrate cleavage is based on the fluorescence signal reading of tube 8. This reading reflects the fluorescence from the 500 pmol standard, which indicates 50% cleaved product, since the amount of the substrate in the reaction is 1,000 pmol.

To calculate the percentage of substrate cleavage in the test sample subtract the FU value of the blank (reaction 1) and follow the equation below.

S = amount (pmol) of fluorescent product in the test sample (reaction 4) as obtained from the standard curve.

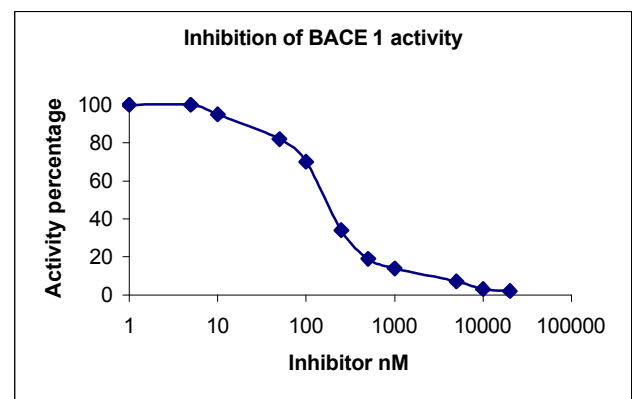
$$\% \text{ cleavage} = \frac{S \text{ (pmol)} \times 50}{500 \text{ (pmol)}}$$

Assay of BACE1 inhibitors

Enzyme activity inhibition reactions were set using reactions 1–3 described in Table 1 (i.e., blank, enzyme activity reaction, and inhibition reaction tubes). Reaction 3 was expanded to include a few wells with different concentrations of the inhibitor ([Asn⁶⁷⁰, Sta⁶⁷¹, Val⁶⁷²]-Amyloid β/A4 Precursor Protein 770 Fragment 662-675 ammonium salt, Catalog Number A1847) and the reaction was performed at 37 °C for 2 hours.

The effect of inhibitor concentration (nM) on enzyme activity is shown in Figure 2.

Figure 2.
Inhibition of BACE1 activity by a specific inhibitor (Catalog Number A1847).



Troubleshooting

The assay is designed so that significant signals are obtained in 1–2 hours.

Several parameters that can affect the signal should be taken into consideration:

1. The sensitivity of the fluorometer used: if the signal is too low, the researcher can increase the amount of substrate and enzyme, or perform the reaction for a longer period of time (e.g., 4–6 hours).

The kit was tested using Perkin Elmer luminescence spectrometer LS50B that can detect 30–500 pmol of product.

2. Effect of enzyme buffer: Perform preliminary tests to confirm the enzyme activity or its detection will not be affected by the volume of sample enzyme buffer in the reaction mixture. High salt concentrations may interfere with the enzyme activity detection.
 - a. Use CelLytic™ M (Catalog Number C2978) to extract sample enzyme from mammalian cells and CelLytic MT (Catalog Number C3228) to extract from tissues.
 - b. The amount of CelLytic MT in the reaction mixture should not exceed 20 µl. Increased enzyme concentration in these extraction buffers may reduce the signals.
 - c. If the enzyme is a secreted enzyme, cells should be grown in a colorless, serum-free medium for a day before the test is to be run, if possible.

3. Effect of solvents: Solvents can decrease enzyme activity and the fluorescence signal. Evaluate each solvent used to determine its effect on enzyme activity and the fluorescence signal.
4. Solvent concentration: The final concentration of DMSO, methanol, or ethanol added should not exceed 4%.

References

1. Citron, M., Beta-secretase as a target for the treatment of Alzheimer's disease. *J. Neurosci. Res.*, **70**, 373-379 (2002).
2. Hong, L., *et al.*, Memapsin 2 (beta secretase) as a therapeutic target. *Biochem. Soc. Trans.*, **30**, 530-534 (2002).
3. Rochette, M. J., and Murphy, M. P., Gamma-secretase: substrates and inhibitors. *Mol. Neurobiol.*, **26**, 81-95 (2002).
4. Roberds, S. L., *et al.*, BACE knockout mice are healthy despite lacking the primary beta-secretase activity in brain: implications for Alzheimer's disease therapeutics. *Hum. Mol. Genet.*, **10**, 1317-1324 (2001).

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