BACE Inhibitor Screening Assay Kit

Item No. 600070

Customer Service 800.364.9897 * **Technical Support** 888.526.5351
www.caymanchem.com

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Materials Supplied

Kit will arrive packaged as a -80°C kit. For best results, remove components and store as stated below.

Item Number	Item	Quantity/Size	Storage
600071	BACE Assay Buffer (10X)	1 vial/3 ml	-20°C
600072	BACE (human recombinant)	1 vial/30 µl	-80°C
600073	BACE Assay Substrate	1 vial/300 µl	-80°C
400017	96-Well Plate (black)	1 plate	Room temperature
400012	96-Well Cover Sheet	1 cover	Room temperature

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: This product is for laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman's BACE Inhibitor Screening Assay Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

E-Mail: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section, on page 3, and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A fluorescence plate reader or a fluorometer capable of measuring fluorescence using excitation and emission wavelengths of 335-345 and 485-510 nm, respectively
2. Adjustable pipettes and a repeating pipettor
3. A source of UltraPure water (Milli-Q or HPLC-grade water)

INTRODUCTION

Background

Accumulation of the β-amyloid peptide (Aβ) in the brain is implicated as the primary cause of neurodegeneration and progression of Alzheimer's disease (AD).[1] The β-amyloid peptide is derived from sequential proteolytic cleavage of the amyloid precursor protein (APP) by β- and γ-secretases. Initial cleavage by β-secretase (BACE; β-site of APP cleaving enzyme), a membrane anchored aspartic protease, generates a soluble N-terminal fragment and a membrane-associated C-terminal fragment.[2] The C-terminal fragment then undergoes proteolysis by γ-secretase to give the Aβ peptide.

BACE has been shown to be the major β-secretase and a promising therapeutic target as this protease initiates the first step in Aβ production.[1] Inhibition of BACE activity could potentially block the entire cascade of Alzheimer's disease pathogenesis. In addition, BACE deficient mice do not generate Aβ peptide. In transgenic murine models of AD driven by Aβ overproduction, BACE deficiency rescued memory deficits and cholinergic dysfunction.[3] Additionally, the fact that β-secretase is an aspartic protease has also raised the hope that its therapeutic inhibitor can be as successful as that against HIV protease.

About This Assay

Cayman's BACE Inhibitor Screening Assay Kit provides a convenient method for screening human BACE inhibitors. The assay utilizes a synthetic Swedish mutant APP peptide (EVNLDAEF) that has been linked to a fluorophore (EDANS) at one end and to a quenching agent (Dabcyl) at the other.[4] After cleavage by BACE, the product (peptide-EDANS) is brightly fluorescent and can be easily analyzed using a fluorescence plate reader or a fluorometer with excitation wavelengths of 335-345 nm and emission wavelengths of 485-510 nm.

PRE-ASSAY PREPARATION

NOTE: Water used to prepare all reagents and buffers must be deionized. Glass distilled water, HPLC-grade water, and sterile water (for injections) are adequate for this kit. UltraPure water may be purchased from Cayman (Item No. 400000).

Reagent Preparation

1. BACE Assay Buffer (10X)

Dilute the vial of BACE Assay Buffer (10X) concentrate (Item No. 600071) with 27 ml of HPLC-grade water. This diluted Assay Buffer (50 mM sodium acetate, pH 4.5) should be used in the assay and for dilution of BACE. When stored at -80°C, this diluted Assay Buffer is stable for at least six months.

2. BACE (human recombinant)

This vial (Item No. 600072) contains a 30 µl solution of human recombinant BACE. Prior to assaying, thaw the enzyme on ice and dilute 30 µl of enzyme with 270 µl of diluted Assay Buffer. This is sufficient enzyme for the full 96-well plate. If not utilizing the entire plate, adjust the amount of diluted enzyme accordingly by diluting the enzyme 1:10 with assay buffer before use. The diluted enzyme is stable for four hours on ice. Prepare aliquots of the remainder of the undiluted enzyme and store at -80°C.

3. BACE Assay Substrate

The vial (Item No. 600073) contains 300 µl of a 400 µM solution of H-RE(EDANS) EVNLDAEFK (Dabcyl)R-OH in DMSO. The substrate is ready to use as supplied. *NOTE: The K_m value for the substrate is 9 µM for human recombinant BACE. The final concentration of substrate in the assay as described below is 10 µM. This concentration may be reduced with DMSO at the user's discretion, particularly when complete inhibition curves are required for IC_{50} value or K_i determination. For competitive inhibitors, the IC_{50} is dependent upon the substrate concentration and should be reported when publishing the experimental results.*

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. However, it is necessary to have three wells designated as background wells and three wells designated as 100% Initial Activity wells. We suggest that each Inhibitor sample be assayed in triplicate and you record the contents of each well on the template sheet provided on page 15. A typical layout of samples and inhibitors to be measured in triplicate is given below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	BW	BW	BW	7	7	7	15	15	15	23	23	23
B	A	A	A	8	8	8	16	16	16	24	24	24
C	1	1	1	9	9	9	17	17	17	25	25	25
D	2	2	2	10	10	10	18	18	18	26	26	26
E	3	3	3	11	11	11	19	19	19	27	27	27
F	4	4	4	12	12	12	20	20	20	28	28	28
G	5	5	5	13	13	13	21	21	21	29	29	29
H	6	6	6	14	14	14	22	22	22	30	30	30

BW - Background Wells
A - 100% Initial Activity Wells
1-30 - Inhibitor Wells

Figure 1. Inhibitor screening plate format

Pipetting Hints

- Use different tips to pipette the buffer, substrate, inhibitors, and enzyme.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 100 µl in all the wells.
- It is not necessary to use all the wells on the plate at one time.
- The assay temperature is 25°C. Pre-warm the Assay Buffer to room temperature before assaying.
- It may be necessary to assay inhibitors at several concentrations to determine an effective concentration.
- We recommend assaying samples in triplicate, but it is the user's discretion to do so.
- 30 inhibitor samples can be assayed in triplicate or 45 in duplicate.

Performing the Assay

NOTE: See Table 1; Pipetting summary below for additional clarity.

1. **Background Wells** - add 95 µl of Assay Buffer and 2.5 µl of solvent (which ever solvent you dissolved your inhibitor in) to three wells.
2. **100% Initial Activity Wells** - add 92.5 µl of Assay Buffer, 2.5 µl of BACE, and 2.5 µl of solvent (which ever solvent you dissolved your inhibitor in) to three wells.
3. **Inhibitor Wells** - add 92.5 µl of Assay Buffer, 2.5 µl of BACE , and 2.5 µl of inhibitor* to three wells.
4. Initiate the reactions by adding 2.5 µl of Substrate to all wells being used. Carefully shake the microwell plate for 10 seconds to mix and cover with the plate cover. Incubate for 40 minutes at room temperature. (This can also be read in the kinetic mode for 40 minutes if desired.)
5. Remove the plate cover and read the fluorescence using excitation wavelengths of 335-345 nm and emission wavelengths of 485-510 nm.

*Inhibitors can be dissolved in methanol, DMSO, or ethanol and should be added to the assay in a final volume of 2.5 µl. In the event that the effective concentration of inhibitor is completely unknown, we recommend that several dilutions of the inhibitor be analyzed.

Well	Assay Buffer	Solvent	Inhibitor	BACE	Substrate
Background	95.0 µl	2.5 µl	-	-	2.5 µl
100% Initial Activity	92.5 µl	2.5 µl	-	2.5 µl	2.5 µl
Inhibitor	92.5 µl	-	2.5 µl	2.5 µl	2.5 µl

Table 1. Pipetting summary

ANALYSIS

Calculations

1. Determine the average fluorescence (AF) of the background wells, 100% Initial Activity wells, and each of the inhibitors.
2. Subtract the background AF from the 100% Initial Activity and Inhibitor AFs.
3. Use the following equation to calculate the percent activity remaining:

$$\% \text{ Initial Activity} = \left[\frac{\text{Inhibited AF}}{\text{100\% Initial Activity}}\right] \text{ x } 100\%$$

4. If multiple concentrations of inhibitor are tested, graph the percent initial activity as a function of the Inhibitor concentration to determine the IC_{50} value (concentration at which there is 50% inhibition). An example of human recombinant BACE inhibitor (IC_{50} = 25 nM)[5] is shown in Figure 2, on page 11.

Performance Characteristics

Precision:

Intra-assay coefficient of variation = 3.5% (n = 24). Inter-assay coefficient of variation = 7% (n = 5).

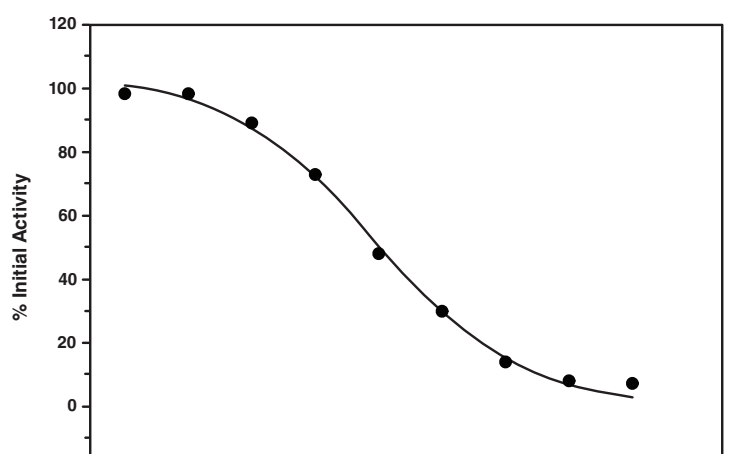

Figure 2. Inhibition of human recombinant BACE by a potent inhibitor (IC_{50} = 25 nM)

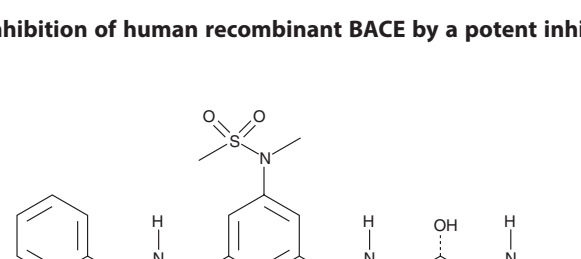

Figure 3. Potent inhibitor used in Figure 2

Interferences

The following reagents were tested in the assay for interference:

Reagent		Will Interfere (Yes or No)
Buffers	Borate	No
	Phosphate	No
	Tris	No
Detergents/Chelators	EDTA (≤1 mM)	No
	EGTA (≤1 mM)	No
	Triton 20 (≤1%)	No
	Triton X-100 (≤1%)	No
Solvents	DMSO (10 µl)	No
	Ethanol (10 µl)	No
	Methanol (10 µl)	No
Others	BSA (≤0.1%)	No
	Glycerol (≤5%)	No

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
No fluorescence above background is seen in the Inhibitor wells	A. Enzyme or substrate was not added to the well(s) B. Inhibitor concentration is too high resulting in complete loss of enzyme activity	A. Make sure to add all components to the wells B. Reduce the concentration of the inhibitor and re-assay
No inhibition seen with inhibitor	The inhibitor concentration is not high enough or the compound is not an inhibitor of the enzyme	Increase the inhibitor concentration and re-assay

References

1. Hong, L., Turner, R.T.I., Koelsch, G., *et al.* Memapsin 2 (β-secretase) as a therapeutic target. *Biochem. Soc. Trans.* **30(4)**, 530-534 (2002).
2. Grüninger-Leitch, F., Schlatter, D., Küng, E., *et al.* Substrate and inhibitor profile of BACE (β-secretase) and comparison with other mammalian aspartic proteases. *J. Biol. Chem.* **277(7)**, 4687-4693 (2002).
3. Pietrak, B.L., Crouthamel, M.-C., Tugusheva, K., *et al.* Biochemical and cell-based assays for characterization of BACE-1 inhibitors. *Anal. Biochem* **342**, 144-151 (2005).
4. Mancini, F., Naldi, M., Cavrini, V., *et al.* Multiwell fluorometric and colorimetric microassays for the evaluation of β-secretase (BACE-1) inhibitors. *Anal. Bioanal. Chem.* **388**, 1175-1183 (2007).
5. Stachel, S.J., Coburn, C.A., Steele, T.G., *et al.* Structure-based design of potent and selective cell-permeable inhibitors of human β-secretase (BACE-1). *J. Med. Chem.* **47(26)**, 6447-6450 (2004).

Related Products

Renin Inhibitor Screening Assay Kit - Item No. 10006270
Renin (human recombinant) - Item No. 10006217

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Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

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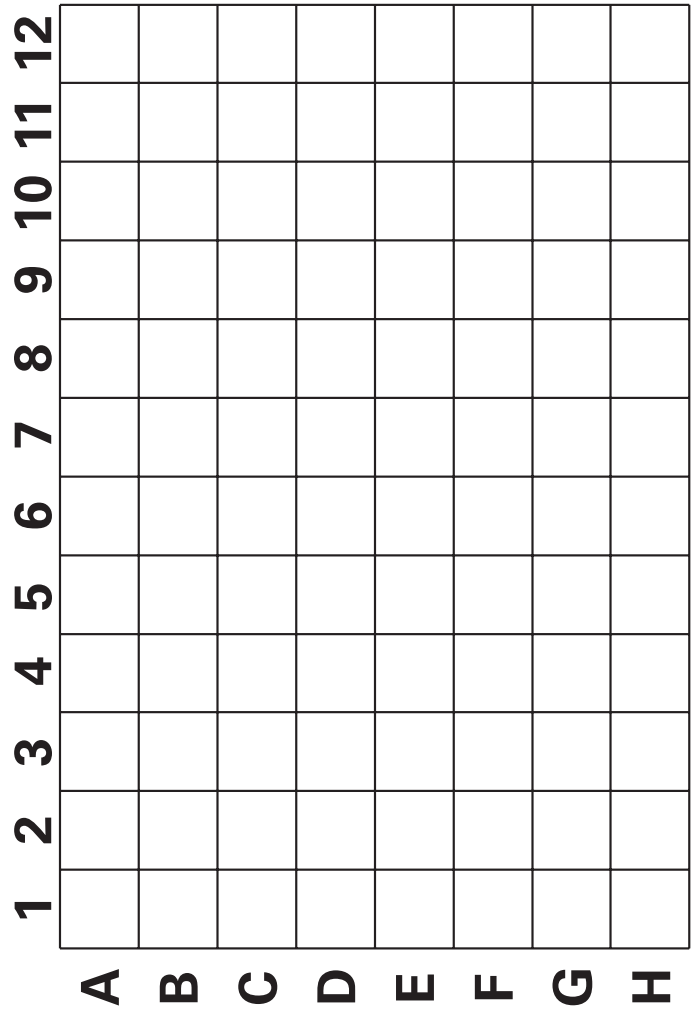
Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.



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