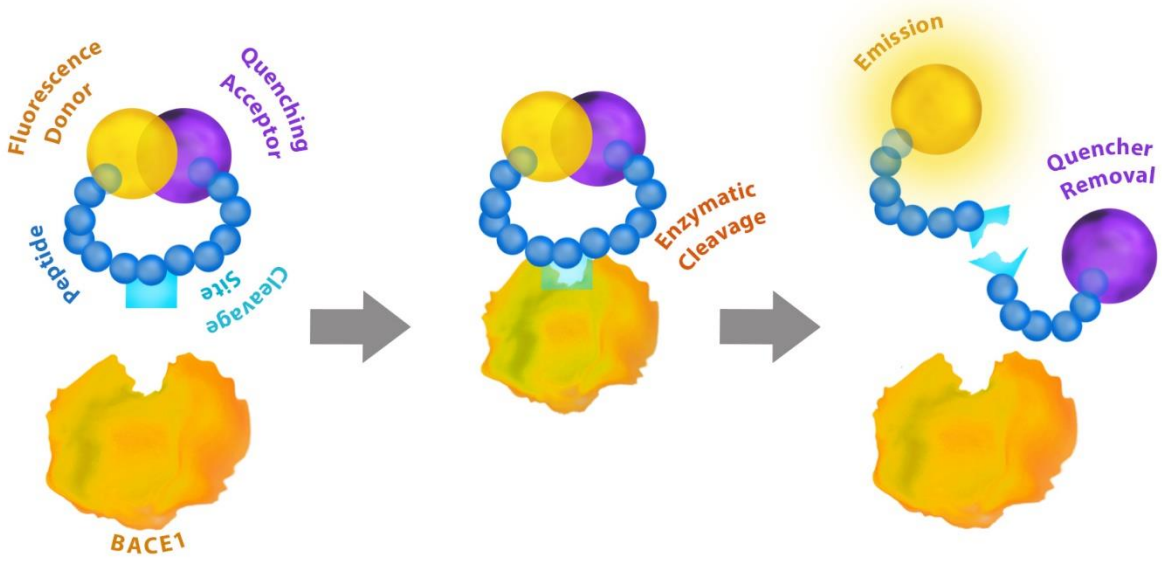
Description

The BACE1 Assay Kit is designed to measure BACE1 activity for screening and profiling applications using a labeled peptide substrate in a homogeneous fluorogenic assay. This Assay Kit comes in a convenient 96-well format, with enough purified recombinant human BACE1 enzyme, BACE1 peptide substrate, and assay buffer for 100 enzyme reactions.

The peptide substrate contains a fluorescence Donor that is quenched by an Acceptor when the peptide is intact. Upon BACE1-mediated cleavage the two peptide fragments pull apart, releasing the highly fluorescent peptide fragment from quenching. Therefore, the fluorescence intensity increases proportionally to the activity of BACE1.



Background

Bace1 (β-secretase 1, also known as beta-site amyloid precursor protein cleaving enzyme 1 and ASP2, GenBank Accession No. NM_012104.4) is an aspartic protease involved in the processing of the Amyloid precursor protein (APP). Cleavage of APP by BACE1 followed by γ-secretase results in β-amyloid peptide production, which ultimately leads to toxic Amyloid β accumulation. Amyloid β aggregation may play a critical role in Alzheimer's disease pathogenesis, suggesting that BACE1 could be a potential therapeutic target in this disease.

Assay Kit Format

Fluorogenic

Applications

1. Study enzyme kinetics
2. Screen for small molecular inhibitors for drug discovery and High Throughput (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
71657	Human BACE1, His-tag*	20 µg	-80°C
	BACE1 Assay Buffer	12 ml	4°C
	BACE1 peptide substrate	100 µl	-80°C
	96-well plate, black	1	Room Temperature

**** The concentration of protein is lot-specific and will be indicated on the tube containing the protein***

Materials Required but Not Supplied

Microplate reader capable of reading fluorescence
Adjustable micropipettor and sterile tips

Storage Conditions

This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Assay Protocol

All samples and controls should be tested in duplicate.

1. Thaw **BACE1 assay buffer** and **BACE1 peptide substrate**.
   Once the BACE1 assay buffer is thawed, it can be stored at 4°C. Please be sure to bring the assay buffer at room temperature before use in the assay.
2. Add 90 µl of **BACE1 assay buffer** only in the Blank wells.
3. Prepare a Master Mix (70 µl per well): N wells x (69 µl **BACE1 assay buffer** + 1 µl **BACE1 peptide substrate**). Add 70 µl to every well except the "Blank" wells.
   Note: Immediately after adding the master mix, cover the plate with aluminum foil and try to keep the plate in the dark as much as possible.
4. Add 10 µl of Inhibitor dilutions of each well labeled as "Test Inhibitor". For the "Positive Control" and "Blank", add 10 µl of a Diluent Solution without inhibitor (the diluent solution consists of the same concentration of diluent used to prepare the inhibitor, for example DMSO diluted in assay buffer).
   Note: the BACE1 assay kit can be used with up to 1% DMSO final concentration.
5. Thaw **BACE1** enzyme on ice. Briefly spin the tube containing the enzyme to recover the full contents of the tube. Dilute the enzyme to ~7.5-10 ng/µl with BACE1 assay buffer. Store remaining undiluted enzyme in aliquots at -80°C.
   Note: **BACE1** enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do

not re-use the diluted enzyme.

6. Initiate the reaction by adding 20 µl of diluted **BACE1** enzyme to the wells designated “Positive Control” and "Test Inhibitor Control".

Component	Blank	Positive Control	Test Inhibitor
Master Mix	90 µl	70 µl	70 µl
Test Inhibitor	-	-	10 µl
Diluent Solution (no inhibitor)	10 µl	10 µl	-
BACE1 (7.5-10 ng/µl)	-	20 µl	20 µl
Total	100 µl	100 µl	100 µl

7. Read the plate using a plate reader capable of detecting fluorescence

Continuous fluorescence reading is highly recommended, although end point reading could also be applicable.

For continuous reading, set up the microplate reader as “Kinetic mode” and read fluorescence for 20 min at Ex 320 nm/Em 405 nm. The slope represents BACE1 activity

For end point reading, read the fluorescence at Ex 320 nm/Em 405 nm immediately after adding BACE1 and read again after incubating the plate for 20 minutes at room temperature).

ΔF=Ft – F0 (Ft: Fluorescence at time t, F0: Fluorescence at time zero) indicates BACE1 activity in end point reading.

Example Results

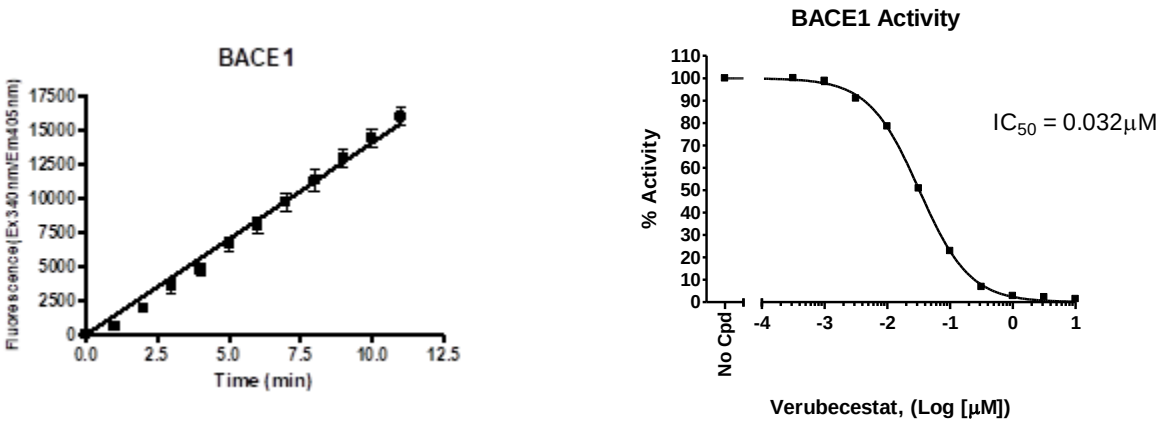


Figure 1: BACE1 activity as a function of time and in the presence of increasing concentrations of inhibitor verubecestat. Continuous monitoring of BACE1 activity (left) and inhibition of BACE1 activity by increasing concentrations of Verubecestat (right) measured using the BACE1 assay kit (Cat. #71656). Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

General Considerations

"Blank" Control: The "Blank" control is important to determine the background fluorescence of the assay. We recommend doing these in duplicate.

"Positive Control": The "Positive Control" is the maximum signal measured in the assay and should not contain test inhibitor.

Trouble Shooting Guide

Visit. bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References

1. Ghosh, A.K., et al. 2012 J. Neurochemistry 120(suppl. 1): 71-83

Related Products

Products	*Catalog #*	*Size*
BACE1, His-Tag Protein	71657	100 µg
Anti-β-Amyloid, Plaque Neutralizing Antibody	71223	100 µg