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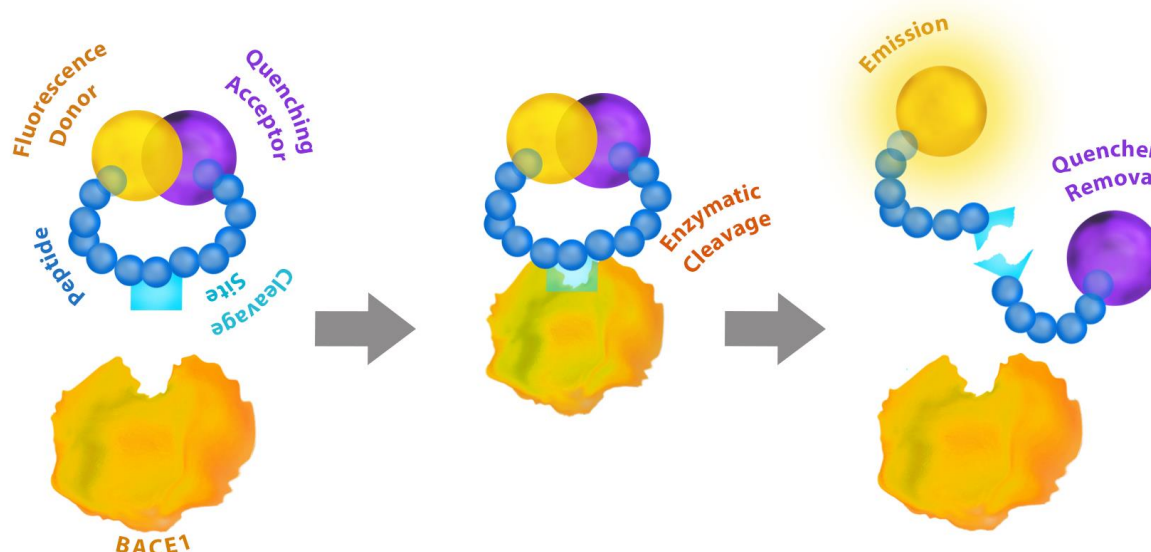
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Data Sheet **BACE1 FRET Assay Kit** Catalog # 71656

DESCRIPTION: Bace1 (β -secretase 1) is an aspartic protease that is 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 toxic A β accumulation. In Alzheimer's disease (AD), it has been widely accepted that A β aggregation plays a critical role in AD pathogenesis, suggesting that BACE1 could be a potential target to treat AD. The *BACE1 FRET Assay Kit* is designed to measure BACE1 activity for screening and profiling applications based on fluorescence resonance energy transfer (FRET) using a labeled peptide substrate (below). The BACE1 FRET Assay Kit comes in a convenient 96-well format, with enough purified recombinant human BACE1 enzyme, BACE1 FRET peptide substrate, and assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
71657	BACE1	20 μ g	-80°C	Avoid multiple freeze/thaw cycles!
	BACE1 assay buffer	12 ml	4°C	
	BACE1 FRET peptide substrate	100 μ l	-80°C	
	96-well plate, black	1	Room Temp.	



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MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

Microplate reader capable of reading fluorescence
Adjustable micropipettor and sterile tips

APPLICATIONS: Useful for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: Up to 6 months when stored as recommended.

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

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

- 1) Thaw **BACE1 assay buffer** and **BACE1 FRET peptide substrate**.
(Once **BACE1 assay buffer** is thawed, it can be stored at 4°C. Please be sure that **BACE1 assay buffer** is at room temperature before use in the assay)
- 2) Prepare the master mixture (70 µl per well): N wells x (69 µl **BACE1 assay buffer** + 1 µl **BACE1 FRET peptide substrate**). Add 70 µl to every well.

	Positive Control	Test Inhibitor	Blank
BACE1 assay buffer	69 µl	69 µl	89 µl
BACE1 FRET peptide substrate	1 µl	1 µl	1 µl
Test Inhibitor	-	10 µl	-
Inhibitor Buffer (no inhibitor)	10 µl	-	10 µl
BACE1 (7.5-10 ng/µl)	20 µl	20 µl	-
Total	100 µl	100 µl	100 µl

Immediately after adding the master mix, cover plate with aluminum foil, and try to keep the plate in the dark as much as possible.

- 3) Add 10 µl of Inhibitor solution of each well labeled as "Test Inhibitor". For the "Positive Control" and "Blank", add 10 µl of the same solution without inhibitor. Note: the BACE1 FRET assay kit can be used with up to 1% DMSO final concentration.
- 4) To the wells designated as "Blank", add 20 µl of BACE1 assay buffer.

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- 5) Thaw BACE1 enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Calculate the amount of BACE1 required for the assay and dilute 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 thawed aliquots or diluted enzyme.*
- 6) Initiate reaction by adding 20 μl of diluted BACE1 enzyme to the wells designated "Positive Control" and "Test Inhibitor Control".
- 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.

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)

- 8) For continuous reading, the slope represents BACE1 activity while $\Delta F = F_t - F_0$ (F_t : Fluorescence at time t, F_0 : Fluorescence at time zero) indicates BACE1 activity in end point reading.

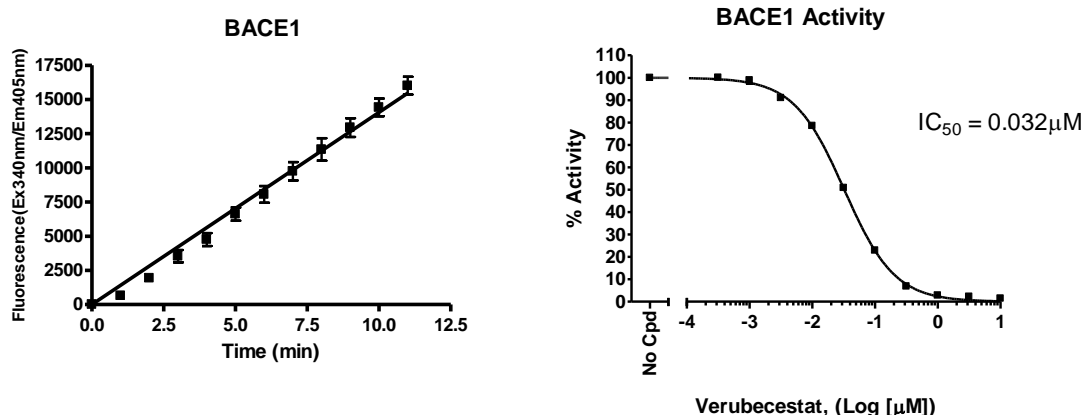
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Example of Assay Results:



Continuous monitoring of BACE1 activity (left) and inhibition of BACE1 activity by Verubecestat (right) measured using the BACE1 FRET assay kit (Cat. #71656). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

<u>Product Name</u>	<u>Catalog #</u>	<u>Size</u>
BACE1, His-Tag Protein	71657	100 μg
Anti-β-Amyloid, Plaque Neutralizing Antibody	71223	100 μg
Tau-316 Protein	90327	20 μg
Tau-352 Protein	90328	20 μg
Tau-381 Protein	90329	20 μg
Tau-383 Protein	90311	20 μg
Tau-383 (N352H) Protein	90310	20 μg
Tau-410 Protein	90312	20 μg
Tau-412 Protein	90313	20 μg
Tau-441 (1-421) Protein	90314	20 μg
Tau-441 (A152T) Protein	90341	20 μg
Tau-441 (dK280) Protein	90342	20 μg
Tau-441 (dN296) Protein	90343	20 μg
Tau-441 (G272V) Protein	90344	20 μg
Tau-441 (K257T) Protein	90345	20 μg
Tau-441 (L266V) Protein	90346	20 μg
Tau-441 (N279K) Protein	90347	20 μg
Tau-441 (P301L) Protein	90348	20 μg

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