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β-Secretase (BACE1) Activity Assay Kit II (Fluorometric)

rev 08/19

(Catalog # K388-100; 100 assays; Store at -80°C)

I. Introduction:

β-Secretase (EC 3.4.23.46), also called BACE1 (β-site of APP cleaving enzyme) or memapsin-2 (membrane-associated aspartic protease), is an aspartic-acid protease. Along with γ-secretase, BACE1 cleaves the APP (Membrane-anchored amyloid precursor protein) and generates soluble amyloid-β (Aβ). Accumulation of Aβ is the key event for pathogenesis of Alzheimer's disease, thus monitoring and targeting BACE1 activity are important for the study and development of therapeutic strategies that could alleviate/cure Alzheimer's disease. Recent studies have shown that inhibiting BACE1 activity with synthetic compounds helped to slow or arrest Alzheimer's disease symptoms. BioVision's BACE1 Assay Kit II provides a fast and easy method for measuring BACE1 activity in a variety of samples. In this kit, a secretase-specific peptide is conjugated with two reporter molecules, EDANS and DABCYL. In the uncleaved form, the fluorescent emissions from EDANS are quenched by the physical proximity of the DABCYL moiety. Cleavage of the peptide by BACE1 separates EDANS and DABCYL groups allowing for the release of a strong fluorescent signal (Ex/Em= 345/500 nm). The level of secretase activity in samples is proportional to the level of fluorescence intensity. The assay is simple, sensitive, high–throughput adaptable and can detect BACE1 activity as low as 0.2 µU per sample.

$$\text{Quenched Fluorescent Substrate} \xrightarrow{\text{BACE1}} \text{Cleaved Fluorescent Product (Ex/Em= 345/500 nm)}$$

II. Application:

- Measurement of BACE1 activity in various tissues/cells
- Analysis and mechanistic study the Alzheimer's disease
- Screening potential therapeutic compounds for Alzheimer's disease

III. Sample Type:

- Animal tissues: Brain and Liver, etc.
- Cell culture: Adherent or Suspension Cells

IV. Kit Contents:

Components	K388-100	Cap Code	Part Number
BACE1 Extraction Buffer	25 ml	NM	K388-100-1
BACE1 Assay Buffer	10 ml	WM	K388-100-2
BACE1 Substrate (in DMSO)	200 µl	Amber	K388-100-3
BACE1 Positive Control	20 µl	Red	K388-100-4
EDANS Standard (100 µM)	100 µl	Yellow	K388-100-5
BACE1 Inhibitor Control (in DMSO)	100 µl	Blue	K388-100-6

V. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

VI. Storage, Handling and Reagent Preparation:

Store kit at -20°C, protected from light. Warm BACE1 Extraction Buffer and BACE1 Assay Buffer to room temperature before use. Briefly centrifuge small vials prior to opening.

- **BACE1 Extraction Buffer and BACE1 Assay Buffer:** Warm to room temperature (RT) before use. Store at 4°C.
- **BACE1 Substrate, EDANS Standard and BACE1 Inhibitor Control:** Thaw to RT. Ready to use. Store at -20°C. Use within two months.
- **BACE1 Positive Control:** Keep on ice while in use. Aliquot and **store immediately at -80°C**. Use within two months.

VII. BACE1 Activity Assay Protocol:

1. Sample Preparation:

Cells or tissue lysate: Rapidly homogenize tissue (10 mg) or cells (10 x 10^6) with 500 µl ice-cold **BACE1 Extraction Buffer**, and place on ice for 10 min. Centrifuge at 10,000 x *g* for 5 min at 4°C and collect the supernatant. Use saturated ammonium sulfate to precipitate proteins and remove interferences such as small molecules: Aliquot homogenized samples (100 µl) to a clean centrifuge tube, add saturated 4.32 M ammonium sulfate (BioVision Cat. # 7096) bringing saturation to 65% (1 volume of sample + 2 volumes of 4.32 M ammonium sulfate). Place on ice for 30 mins. Spin down samples at 10,000 x *g* at 4°C for 10 min, discard supernatant, and resuspend the pellet back to the original volume with **BACE1 Assay Buffer**. Add 2-50 µl Samples into a 96 well white plate. Adjust final volume to 50 µl with BACE1 Assay Buffer. **BACE1 Positive Control:** Add 2-5 µl of BACE1 Positive Control into the wells and adjust final volume to 50 µl with BACE1 Assay Buffer.

Notes:

a. For Unknown Samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

b. For Unknown Samples or Samples exhibiting high background, prepare parallel Sample well(s) as Sample Background Controls.

2. EDANS Standard Curve: Dilute EDANS Standard to 10 µM by adding 10 µl of 100 µM EDANS to 90 µl BACE1 Assay Buffer. Add 0, 2, 4, 6, 8 and 10 µl of diluted 10 µM EDANS Standard into a series of wells to generate 0, 20, 40, 60, 80 and 100 pmol/well of EDANS Standard. Adjust volume to 100 µl/well with BACE1 Assay Buffer.

3. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Mix containing:

	Reaction Mix	Background Control Mix*
BACE1 Assay Buffer	48 µl	47 µl
BACE1 Substrate	2 µl	2 µl
BACE1 Inhibitor Control	----	1 µl

Add 50 µl of the Reaction Mix to each well containing Test Samples and BACE1 Positive Control well(s).
* For Unknown Samples or Samples with high background, add 50 µl of Background Control Mix in a duplicate well(s).

4. Measurement: Measure the plate immediately at Ex/Em= 345/500 nm in a kinetic mode for 10-60 min at 37°C.

Note: Incubation time depends on the BACE1 activity in the Samples. We recommend measuring absorbance in kinetic mode, and choosing two time points (t_1 & t_2) in the linear range to calculate the BACE1 activity of the Samples. If low activity is expected, longer incubation times may be needed. The EDANS Standard Curve can be read in Endpoint mode (i.e. at the end of incubation time).

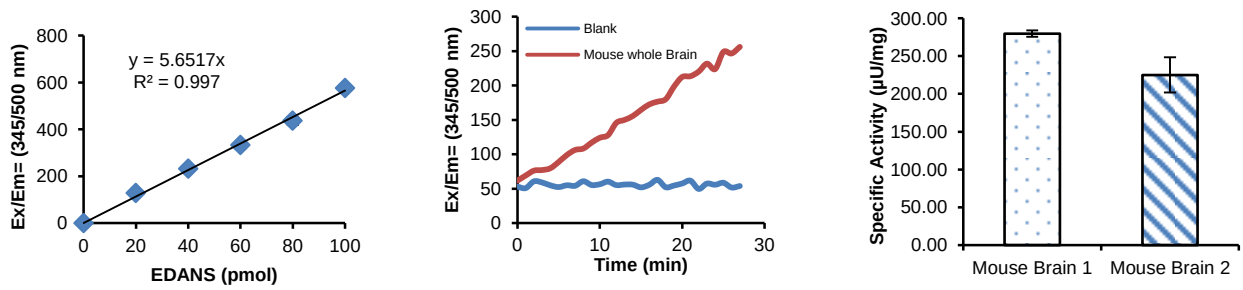
5. Calculation: Subtract the 0 Standard reading from all Standard readings. Plot the EDANS Standard Curve. Correct Sample Background by subtracting the value derived from the Background Control from all Sample readings if necessary. Calculate the BACE1 activity of the Test Sample: $\Delta RFU = RFU_2 - RFU_1$. Apply the ΔRFU to the EDANS Standard Curve to get B pmol of EDANS generated by BACE1 during the reaction time ($\Delta t = t_2 - t_1$).

Sample BACE1 Activity = B/(ΔT X V) x D = pmol/min/µl = µU/µl = mU/ml

Where: **B** is the EDANS amount from Standard Curve (pmol)
ΔT is the reaction time (min)
V is the Sample volume added into the reaction well (µl)
D is the dilution factor

Unit Definition: One unit of EDAN is the amount of enzyme that will generate 1.0 µmol of EDANS per min at pH 4.5 at 37°C.

(a) **(b)** **(c)**



Figures: (a) EDANS Standard Curve. (b) Kinetic measurement of BACE1 activity in mouse whole brain lysate (4 µg). (c) Relative BACE1 Activity was calculated in lysates (4 µg) prepared from mouse whole brain (Sample 1) and mouse whole brain (Sample 2). Assays were performed following the kit protocol.

VIII. RELATED PRODUCTS

β-Secretase Activity Fluorometric Assay Kit (K360)
Human Beta-Secretase Inhibitor Screening Assay Kit (K720)
Pyruvate Dehydrogenase Activity Assay Kit (K679)
Glucose-6-Phosphate Dehydrogenase Assay Kit (K757)
Phosphoglucomutase Assay Kit (K774)
Glucose Dehydrogenase Activity Assay Kit (K786)
Glucose Uptake Colorimetric Assay (K676)
Glucose Uptake Fluorometric Assay (K666)
Glucose-6-Phosphate Assay Kit (657)
Hexokinase Assay Kit (K789)
Phosphoglucose Isomerase Assay Kit (K775)
Glucose-1-Phosphate Assay Kit (K697)

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