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Human β-Secretase (BACE1) Inhibitor Screening Kit (Fluorometric)

rev 06/21

(Catalog # K720-100; 100 assays; Store at -80 °C)

I. Introduction:

β-Secretase (Beta-site amyloid precursor protein (APP) cleaving enzyme 1/BACE1), participates in the initial cleavage of amyloid precursor-APP generating two soluble *N*-terminal and *C*-terminal fragments. Another secretase isozyme, γ-Secretase, cleaves *C*-terminal fragment producing amyloid β. Accumulation of amyloid β in brain results in the formation of amyloid plaques, which contributes in the development of Alzheimer's disease (AD) in humans. Therefore, screening for novel and specific inhibitors of human β-Secretase (BACE1) has become critical in Alzheimer's disease-related drug discovery research. In **BioVision's Human β-Secretase (BACE1) Inhibitor Screening Kit**, human BACE1 cleaves a quenched substrate, specific for BACE1, generating a product with high fluorescence that can be measured at Ex/Em= 345/500 nm. In the presence of BACE1 Inhibitor (control included in this kit), the enzymatic cleavage is impeded and could be used to screen for new inhibitor compounds. The assay is high-throughput adaptable and can be completed in less than 1 hr.

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

Quenched Fluorescent Substrate $\xrightarrow{\text{BACE1 Inhibitor}}$ Lower/No Fluorescent Product (Ex/Em = 345/500 nm)

II. Application:

- Screening/characterizing/studying potential inhibitors of Human β-Secretase (BACE1)

III. Kit Contents:

Components	K720-100	Cap Code	Part Number
BACE1 Assay Buffer	25 ml	WM	K720-100-1
BACE1 Substrate (in DMSO)	200 µl	Amber	K720-100-2
BACE1	20 µl	Red	K720-100-3
BACE1 Inhibitor Control (in DMSO)	10 µl	Blue	K720-100-4

IV. User Supplied Reagents and Equipment:

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

V. Storage Conditions and Reagent Preparation:

Store kit at -80 °C, protected from light. Bring Assay Buffer to RT before use. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

- **BACE1 Substrate, BACE1 Inhibitor Control:** Ready to use. Can be stored at -20 °C. Use within two months.
- **BACE1:** Dilute 1:10 in BACE1 Assay Buffer (2 µl BACE1 + 18 µl BACE1 Assay Buffer). Make as much as needed and avoid repeated free-thaw cycles. Place on ice while in use. Store at -80 °C.

VI. β-Secretase (BACE1) Inhibitor Screening Protocol:

1. **Sample Compounds, Inhibitor Control, and Enzyme Control Preparation:** Dissolve candidate inhibitors into appropriate solvent(s) at high stock concentration to be tested. Dilute to 2X desired test concentration with BACE1 Assay Buffer. Add 50 µl diluted candidate inhibitor or BACE1 Assay Buffer into desired wells for Sample Screen [S], and Enzyme Control [EC] (no inhibitor) respectively. For BACE1 Inhibitor Control (IC): dilute BACE1 Inhibitor Control 50 times by adding 2 µl Inhibitor Control to 98 µl BACE1 Assay Buffer. Add 50 µl of diluted Inhibitor Control into desired well(s).

   Notes:

   High solvent concentration might affect the enzymatic activity. Prepare parallel well(s) as Solvent Control to test the effect of the solvent on enzyme activity (same as EC in presence of final solvent concentration).

2. **BACE1 Enzyme:** Add 2 µl of the diluted BACE1 Enzyme into Sample, Enzyme Control, Solvent control and Inhibitor Control wells. Incubate for 5 min at 25 °C.

3. **Substrate Solution Preparation:** Make enough reagents for the number of assays to be performed. For each well, prepare 50 µl of Substrate solution containing:

BACE1 Assay Buffer	48 µl
BACE1 Substrate	2 µl

   Mix and add 50 µl of Substrate solution into each well. Mix well with gentle shaking.

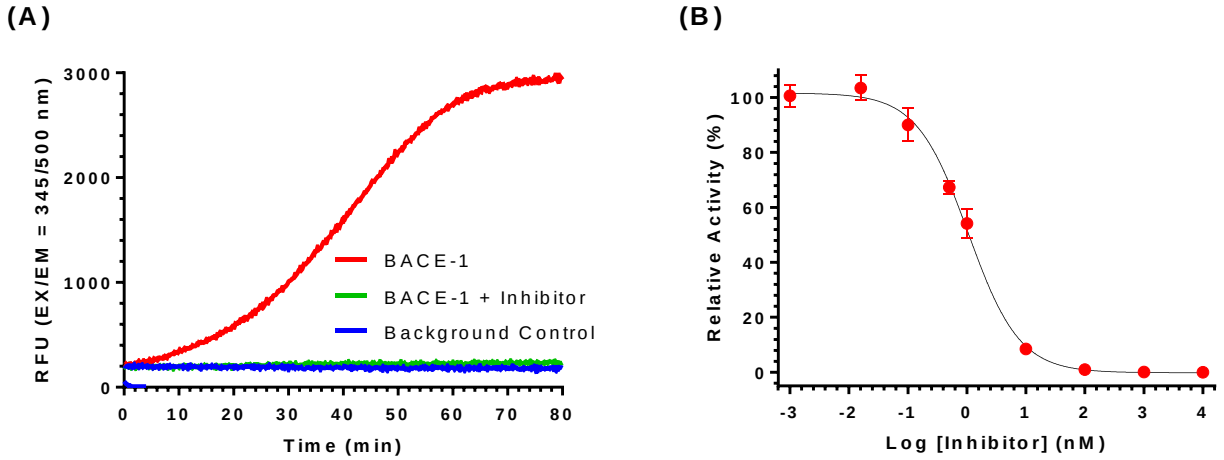
4. **Measurement:** Measure RFU at Ex/Em = 345/500 nm in kinetic mode for 5-60 min at 37 °C. Choose two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding values for the RFU (RFU_1 & RFU_2).

5. **Calculations:** Calculate the slope for all samples, including Enzyme Control [EC], Solvent Control [SC], Sample [S] and Inhibitor Control [IC]. Set [EC] as 100% activity (alternatively, if inhibitory effect is observed by Solvent, set [SC] as 100% activity). *Slope can be calculated by dividing the net ΔRFU (=RFU_2-RFU_1) value by the time Δt (=t_2-t_1).* Calculate % relative inhibition and/or % relative activity using the following equations.

$$\textbf{Relative Activity}\ (\%) = \frac{\textbf{Slope of S}}{\textbf{Slope of EC}}\ \textbf{X 100}$$

$$\textbf{Relative Inhibition}\ (\%) = \frac{\textbf{Slope of EC} - \textbf{Slope of S}}{\textbf{Slope of EC}}\ \textbf{X 100}$$

Where, **Slope of EC** is the slope of Enzyme Control
Slope of S is the slope of Sample Screen



Figures. (A) Progress Curves of BACE Enzyme Control and Inhibitor Control. BACE1 activity was inhibited more than 95% following kit protocols and using Inhibitor Control. **(B)** Inhibition of Human β-Secretase (BACE1) activity by BACE1 Inhibitor (IC_{50} = 1 nM). Assay was performed following the kit protocol.

VII. RELATED PRODUCTS:

Hexokinase Inhibitor Screening Kit (K828)
Factor Xa Inhibitor Screening Kit (K362)
Myeloperoxidase (MPO) Inhibitor Screening Kit (K746)
PCAF Inhibitor Screening Assay (K345)
HMG-CoA Reductase Activity/Inhibitor Screening kit (K588)
SIRT2 Inhibitor Screening Assay Kit (K322)
β-Secretase Activity Fluorometric Assay Kit (K360-100)
AHCY Inhibitor Screening Kit (K326)
Human Calpain 1 Inhibitor Screening Kit (K244)
p300 Inhibitor Screening Kit (K346)
TACE Inhibitor Screening Assay Kit (K366)
SIRT1 Inhibitor/Activator Screening Kit (K325)
SIRT6 Inhibitor Screening Assay Kit (K323)

FOR RESEARCH USE ONLY! Not to be used on humans.