ab283408 – BACE1 Inhibitor Screening Kit (Fluorometric)

For the screening of potential BACE1 inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab283408

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Storage and Stability

On receipt entire assay kit should be stored at -80°C, protected from light. Upon opening, use kit within 6 months.

Materials Supplied

Item	Quantity	Storage Condition
β-Secretase Assay Buffer/BACE1 Assay Buffer	25 mL	-20°C
β-Secretase Substrate/BACE1 Substrate (in DMSO)	200 µl	-20°C
Active β-Secretase/BACE1 Enzyme	200 µl	-80°C
β-Secretase Inhibitor/BACE1 Inhibitor Control (in DMSO)	10 µl	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Multi-well spectrophotometer
- 96-well white plate with flat bottom

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

β-Secretase Assay Buffer/BACE1 Assay Buffer: Bring the β-Secretase Assay Buffer/BACE1 Assay Buffer to room temperature (RT) before use.

β-Secretase Substrate/BACE1 Substrate and β-Secretase Inhibitor/BACE1 Inhibitor: Ready to use. Can be stored at -20°C. Use within two months.

Active β-Secretase/BACE1 Enzyme: Ready to use. Place on ice while in use. Store at -80°C.

Δ Note: *Keep Active β-Secretase/BACE1 Enzyme on ice while performing the assay.*

Assay Protocol

Sample Compounds, Inhibitor Control, and Enzyme Control Preparation:

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

Δ Note: *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).*

Active β-Secretase/BACE1 Enzyme:

1. Add 2 µl of the Active β-Secretase/BACE1 Enzyme into Sample, Enzyme Control, Solvent control, and Inhibitor Control wells. Incubate for 5 minutes at 25°C.

Substrate Solution Preparation:

1. Mix enough solution for the number of assays to be performed. For each well, prepare 50 µl Substrate Solution containing:

Item	Volume
β-Secretase Assay Buffer/BACE1 Assay Buffer	48 µl
β-Secretase Substrate/BACE1 Substrate	2 µl

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

Measurement

1. Measure RFU at Ex/Em = 345/500 nm in kinetic mode for 5-60 minutes at 37°C.
2. Choose any two time points (t1 and t2) in the linear range of the plot and obtain the corresponding values for the RFU (RFU1 and RFU2).

Calculation

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 (=RFU2-RFU1) value by the time Δt (=t2-t1).
Calculate % relative inhibition and/or % relative activity using the following equations.

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

$$\text{\% Relative Inhibition} = \frac{Slope\ of\ EC - Slope\ of\ S}{Slope\ of\ \text{EC}} \times 100$$

Where: Slope of EC is the slope of Enzyme Control
Slope S is the slope of Sample Screen.

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)