β -Secretase Activity Assay Kit

(Catalog #K360-100; 100 assays; Store at -20˚C)

I. Introduction:

β -Secretase has been implicated to be an excellent target for anti-amyloid therapy for the treatment of Alzheimer's disease. The β -Secretase activity Assay Kit provides a convenient fluorescence method for detecting β-secretase activity in biological and purified samples. The assay utilizes a secretase-specific peptide conjugated to 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 secretase physically separates EDANS and DABCYL allowing for the release of a fluorescent signal. The level of secretase enzymatic activity in samples is proportional to the level of fluorescence intensity.

II. Kit Contents:

Component	100 assays	Cap Color	Number
β -Secretase Extraction Buffer	25 ml	NM	K360-100-1
β -Secretase Reaction Buffer (2X)	10 ml	WM	K360-100-2
β -Secretase Substrate (in DMSO)	200 µl	Amber	K360-100-3
Active β -Secretase (Lyophilized)	1 vial	Red	K360-100-4
β -Secretase Inhibitor (in DMSO)	10 µl	Blue	K360-100-5

III. β -Secretase Assay Protocol:

A. General Consideration & Reagent Preparation:

- Reconstitute the lyophilized Active β -Secretase to 10 µl ddH_2O. The enzyme should be refreezed immediately at –70˚C after each use to avoid loss of activity. The enzyme is sufficient for 5 positive control assays (2 µl/assay).
- Assay can be performed directly in a 96-well plate. Nunc F16 Black MaxiSorp™ polystyrene microplate is recommended.

B. Assay Protocol:

1. Collect cells (5 x 10^6 cells/assay) by centrifugation for 5 min at 700x g. Add 0.1 ml of ice-cold Extraction Buffer. For tissue sample, add 2-3X volume of ice-cold Extraction Buffer to tissue sample and homogenize it on ice.
2. Incubate cell lysate on ice for 10 minutes and centrifuge at 10,000x g for 5 minutes. Transfer the supernatant to a new tube and keep on ice. This should yield a lysate with a protein concentration of ~2-4 mg/ml.
3. Add 50 µl cell lysate (~2 - 5 x 10^6 cells or 25 - 200 µg of total protein) to each well in a 96-well plate.

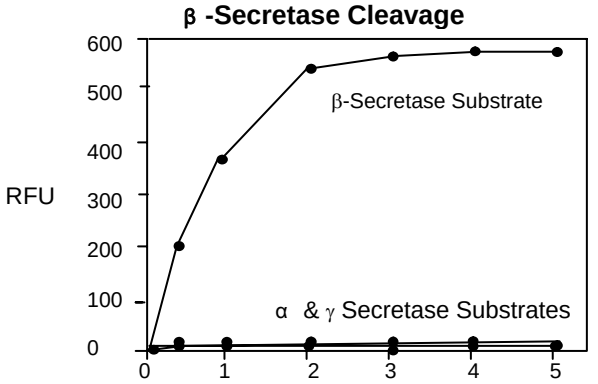
   For positive control assay, add 2 µl of reconstituted Active β -secretase to 50 µl of Extraction Buffer.

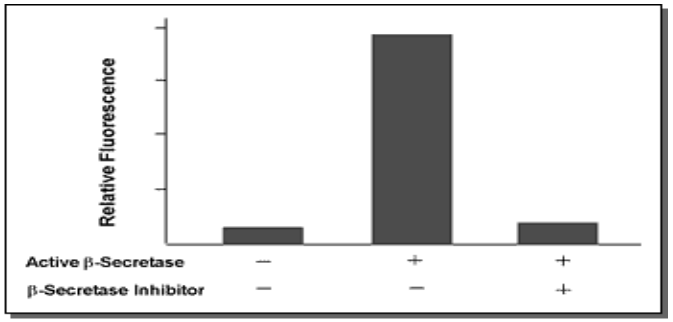
   For negative control assay, add 2 µl of the β -Secretase Inhibitor to 50 µl of Extraction Buffer.
4. Add 50 µl of 2X Reaction Buffer.
5. Add 2 µl of β -Secretase substrate.
6. Cover the plate, tap gently to mix, and incubate in the dark at 37˚C for 1 hour.
7. Read sample in a fluorescence plate reader with Ex. = 335-355 nm and Em. = 495-510 nm.

   Background reading from substrate (without secretase) must be subtracted from all treated and untreated samples before calculating the fold increase in secretase activity (Note: Background reading from substrate can be quite high, due to the nature of such fluorescence quenching assay.)

   β -Secretase activity can be expressed as the Relative Fluorescence Units per µg of protein sample.

   Note: Recombinant β -Secretase exclusively cleaves β -Secretase substrate. It does not cleave α – or γ-Secretase substrates.





IV. Related Products:

- Active β -Secretase
- β -Secretase Inhibitors, Substrates, & Antibodies

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