β-Secretase Activity Assay Kit (Fluorometric)

(Catalog #K360-100; 100 assays; Store at -80 °C)

I. Introduction:

β-Secretase has been implicated to be an excellent target for anti-amyloid therapy for the treatment of Alzheimer's disease. **BioVision's 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	Part 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	20 µl	Red	K360-100-4
β-Secretase Inhibitor (in DMSO)	10 µl	Blue	K360-100-5

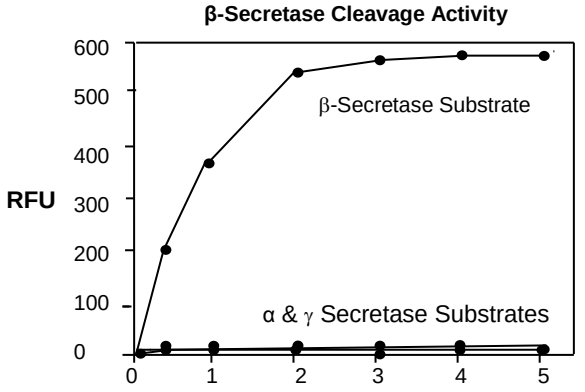
III. β-Secretase Activity Assay Protocol:

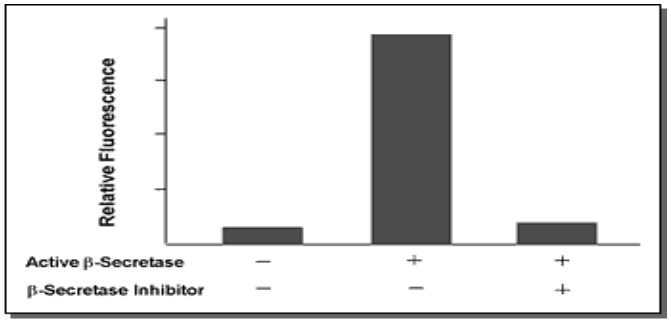
A. General Consideration & Reagent Preparation:

- **Active β-Secretase** is ready to use. The enzyme should be refrozen immediately at -80 °C after each use to avoid loss of activity. The enzyme is sufficient for 5 positive control assays (4 µl/assay).
- Assay can be performed directly in a 96-well white plate with flat bottom.

B. Assay Protocol:

1. Collect cells (5 x 10^6 cells/assay) by centrifugation for 5 min at 700 x *g*. Add 0.1 ml of ice-cold β-Secretase Extraction Buffer. For tissue sample, add 2-3X volume of ice-cold β-Secretase Extraction Buffer to tissue sample and homogenize it on ice.
2. Incubate cell lysate on ice for 10 min and centrifuge at 10,000 x *g* for 5 min. 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 4 µl of Active β-secretase to 50 µl of Extraction Buffer. For Negative Control assay, add 2 µl of the β-Secretase Inhibitor to the 50 µl Sample or Positive Control well.
4. Add 50 µl of 2X Reaction Buffer, gently mix then pre-incubate 20 min at 37 °C **BEFORE ADDING THE SUBSTRATE**).
5. Add 2 µl of β-Secretase substrate.
6. Incubate in the dark at 37 °C for 30 min.
7. Read samples in a fluorescent plate reader with Ex/ Em = 345/500 nm. Background readings obtained from substrate (without the 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.





RELATED PRODUCTS:

- Active β-Secretase
- β-Secretase Inhibitors
- β-Secretase Substrates
- β-Secretase Antibodies

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

Problems	C	
Assay not working	• Use of ice-cold buffer	• Buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range

Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.