Product Information

Beta-Secretase Activity Fluorometric Assay Kit

I. Kit Contents:

Components	K2043-100 100 assays	Cap Color	Part Number
β-Secretase Extraction Buffer	25 ml	NM	K2043-C-1
β-Secretase Reaction Buffer (2X)	10 ml	WM	K2043-C-2
β-Secretase Substrate (in DMSO)	200 µl	Amber	K2043-C-3
Active β-Secretase (Lyophilized)	1 vial	Red	K2043-C-4
β-Secretase Inhibitor (in DMSO)	10 µl	Blue	K2043-C-5

II. Introduction:

β-Secretase is an aspartic-acid protease that plays an important role in Alzheimer's disease and has been an excellent target for the treatment of Alzheimer's disease based on anti-amyloid therapy.

The Beta-Secretase Activity Fluorometric Assay Kit provides a highly sensitive and convenient way for detection of β-secretase activity in various biological and purified samples based on fluorescence method. The assay utilizes a β-secretase-specific peptide substrate conjugated to two molecules EDANS and DABCYL. In the uncleaved form, the fluorescent emissions from EDANS are quenched by the DABCYL moiety because of the physical proximity. Upon cleavage by β-secretase, EDANS is physically separated with DABCYL, which allowing for the release of a fluorescent signal. The activity of β-secretase in samples is proportionally related to the level of fluorescence intensity.

III. General Consideration & Reagent Preparation:

Reconstitute the lyophilized Active β-Secretase by adding 10 µl of ddH_2O. The enzyme should be refrozen immediately at –70℃ after each use to avoid loss of activity. The enzyme is sufficient for 5 positive control assays (2 µl/assay).

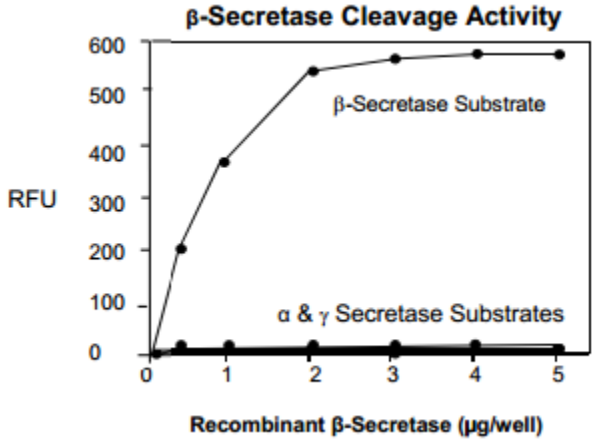
Assay can be performed directly in a 96-well white plate with flat bottom.

IV. 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 the 50 µl Sample or Positive Control well.
4. Add 50 µl of 2X Reaction Buffer. (if using inhibitor, gently mix then pre-incubate 5-10 min at 37℃ before adding substrate.
5. Add 2 µl of β-Secretase substrate.
6. Cover the plate, tap gently to mix, and incubate in the dark at 37℃ for 1 hour.
7. Read samples in a fluorescent plate reader with Ex. 335 - 355 nm and Em. 495-510 nm. Background readings obtained from substrate (withou

t 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.



General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the Nucleotide releasing buffer provided in the kit or refer data sheet for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes

Standard curve	• Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • 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.		

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Our promise

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