

β-Secretase Activity Fluorometric Assay Kit

(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. 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 | 20 µl | Red | K360-100-4 |
| β-Secretase Inhibitor (in DMSO) | 10 µl | Blue | K360-100-5 |

III. β-Secretase Assay Protocol:

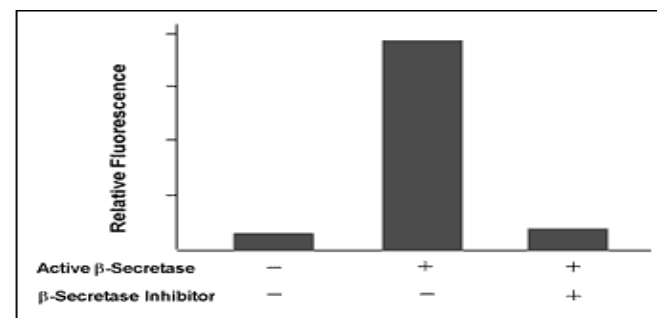
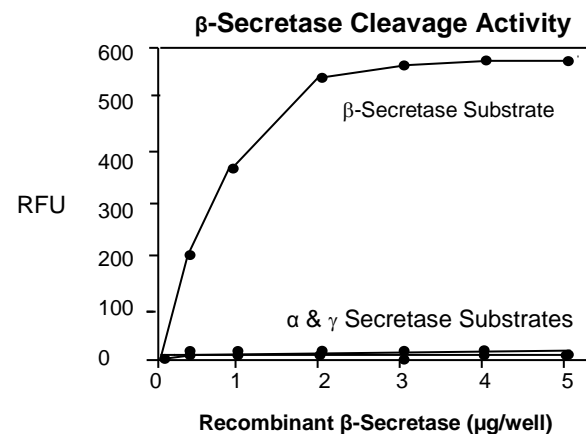
A. General Consideration & Reagent Preparation:

- 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:

- Collect cells (5 x 10⁶ 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.
- 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.
- Add 50 µl cell lysate (~2-5 x 10⁶ 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.
- Add 50 µl of 2X Reaction Buffer, gently mix then pre-incubate 20 min at 37°C **BEFORE ADDING SUBSTRATE**
- Add 2 µl of β-Secretase substrate, pipet up and down to mix well, incubate 10 min at 37°C.
- Cover the plate, tap gently to mix, and incubate in the dark at 37°C for 1 hour.

- Read samples in a fluorescent plate reader with Ex/ Em = 345/500 nm. Background readings obtained 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.



RELATED PRODUCTS:

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

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

GENERAL TROUBLESHOOTING GUIDE:

| Problems | Cause | Solution |
|--|---|---|
| Assay not working | <ul style="list-style-type: none"> • Use of ice-cold buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate | <ul style="list-style-type: none"> • 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 (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates |
| Samples with erratic readings | <ul style="list-style-type: none"> • 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 | <ul style="list-style-type: none"> • Refer data sheet for details about incompatible samples • Use the assay 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 • Use fresh samples or store at correct temperatures until use |
| Lower/ Higher readings in Samples and Standards | <ul style="list-style-type: none"> • 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 | <ul style="list-style-type: none"> • 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 datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly |
| Readings do not follow a linear pattern for Standard curve | <ul style="list-style-type: none"> • Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots | <ul style="list-style-type: none"> • Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • 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 | <ul style="list-style-type: none"> • Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range | <ul style="list-style-type: none"> • 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 |
| <p>Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.</p> | | |