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Product Information

β-Secretase Activity Fluorometric Assay Kit

Catalog Number **MAK237**
Storage Temperature –70 °C

TECHNICAL BULLETIN

Product Description

β-Secretase has been implicated in Alzheimer's disease and may be an excellent target for anti-amyloid therapy for its treatment. 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 emission of a fluorescent signal. The level of secretase enzymatic activity in samples is proportional to the level of fluorescence intensity.

Components

The kit is sufficient for 100 assays in 96 well plates.

β-Secretase Extraction Buffer Catalog Number MAK237A	25 mL
β-Secretase Reaction Buffer (2×) Catalog Number MAK237B	15 mL
β-Secretase Substrate (in DMSO) Catalog Number MAK237C	200 μL
Active β-Secretase Catalog Number MAK237D	20 μL
β-Secretase Inhibitor (in DMSO) Catalog Number MAK237E	10 μL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – white plates are recommended for this assay.
- Fluorescence multiwell plate reader

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge small vials at low speed prior to opening. Use ultrapure water for the preparation of reagents and standards.

Active β-Secretase is a ready to use solution. The enzyme should be refrozen immediately at –70 °C after each use to avoid loss of activity. The enzyme is sufficient for 5 positive control assays (4 μL/assay).

Storage/Stability

Store kit at –70 °C, protected from light. Briefly centrifuge all small vials prior to opening.

Procedure

All samples and standards should be run in duplicate. Read entire protocol before performing the assay.

1. Collect cells (5×10^6 cells/assay) by centrifugation for 5 minutes at 700 × *g*. Add 0.1 mL of ice-cold Extraction Buffer. For tissue sample, add 2–3 volumes 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 10,000 × *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 of cell lysate (2–5 × 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 solution 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 2× Reaction Buffer. If using inhibitor, gently mix, then pre-incubate 20 minutes at 37 °C, (BEFORE ADDING SUBSTRATE).

5. Add 2 μL of β-Secretase substrate. Mix well and incubate 10 minutes at 37 °C.

6. Cover the plate, tap gently to mix, and incubate in the dark at 37 °C for 1 hour.

7. Read samples in a fluorescence plate reader (λ_{ex} = 335–355 nm/ and λ_{em} = 495–510 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

   Notes: 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.

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

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay Not Working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	White plates are recommended for this assay.
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly 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	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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