

ab65357 – Beta-secretase Activity Assay Kit (Fluorometric)

Instructions for Use

For rapid, sensitive and accurate measurement of beta Secretase activity in various samples.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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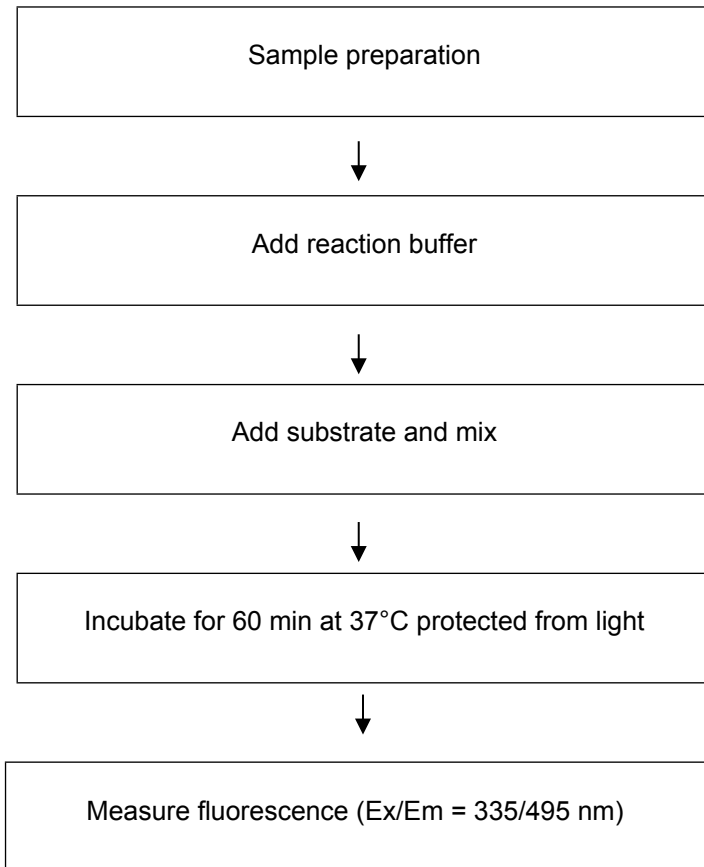
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1. BACKGROUND

β -Secretase (Beta secretase) activity Assay Kit (ab65357) is 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 signal from EDANS is quenched by the physical proximity of the DABCYL moiety. Cleavage of the peptide by β -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.

β -Secretase (Beta secretase, BACE) is responsible for the proteolytic process of the amyloid precursor protein (APP). It cleaves APP at the N-terminus of the A-beta peptide sequence, between residues 671 – 672, leading to the generation and extracellular release of beta-cleaved soluble APP, and a corresponding cell-associated C-terminal fragment which is later released by gamma-secretase. It has been implicated to be an excellent target for anti-amyloid therapy for the treatment of Alzheimer's disease.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -80°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 6 months.**

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
β -Secretase Extraction Buffer	25 mL	-80°C	-80°C
β -Secretase Reaction Buffer/ β -Secretase 2X Reaction Buffer	10 mL	-80°C	-80°C
β -Secretase Substrate/ β -Secretase Substrate (in DMSO)	200 μ L	-80°C	-80°C
Active β -Secretase	20 μ L	-80°C	-80°C
β -Secretase Inhibitor/ β -Secretase Inhibitor (in DMSO)**	10 μ L/100 μ L	-80°C	-80°C

**You may receive 10 μ L or 100 μ L of this component. These sizes are the same formulation and performance.

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- MilliQ water or other type of double distilled water (ddH₂O)
- PBS
- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent microplate reader – equipped with filter for Ex/Em = 335/495 nm)
- 96 well plate: white plate (flat bottom)
- Heat block or water bath
- Dounce homogenizer or pestle (if using tissue)
- Method for protein determination (optional) – we recommend Optiblot Bradford Reagent (ab119216)

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 β -Secretase Extraction Buffer:

Ready to use as supplied. Equilibrate to room temperature before use. Store at -80°C .

9.2 β -Secretase Reaction Buffer/ β -Secretase 2X Reaction Buffer:

Ready to use as supplied. Equilibrate to room temperature before use. Store at -80°C .

9.3 β -Secretase Substrate/ β -Secretase Substrate – in DMSO:

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use.

NOTE: DMSO tends to be solid when stored at -20°C or below, even when left at room temperature, so it needs to melt for few minutes at 37°C . Aliquot substrate so that you have enough volume to perform the desired number of assays. Store at -80°C protected from light. Once thawed, use with two months. Keep on ice while in use.

9.4 Active β -Secretase:

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 ($4\ \mu\text{l}/\text{assay}$).

9.5 β -Secretase Inhibitor/ β -Secretase Inhibitor – in DMSO:

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use.

NOTE: DMSO tends to be solid when stored at -20°C or below, even when left at room temperature, so it needs to melt for few minutes at 37°C . Aliquot inhibitor so that you have enough volume to perform the desired number of assays. Store at -80°C protected from light. Once thawed, use with two months. Keep on ice while in use.

10. SAMPLE PREPARATION

General Sample information:

- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C . When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

10.1 Cell (adherent or suspension) samples:

- 10.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 5×10^6 cells).
- 10.1.2 Wash cells with cold PBS.
- 10.1.3 Resuspend cells in 100 μL of Extraction Buffer.
- 10.1.4 Homogenize cells quickly by pipetting up and down a few times.
- 10.1.5 Incubate cells on ice for 15 – 30 minutes.
- 10.1.6 Centrifuge sample for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.
- 10.1.7 Collect supernatant and transfer to a clean tube.
- 10.1.8 Keep on ice.
- 10.1.9 Optional: measure protein concentration and adjust to 25 – 200 μg per 50 μL with Extraction Buffer.

10.2 Tissue samples:

- 10.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg).
- 10.2.2 Wash tissue in cold PBS.

- 10.2.3 Resuspend tissue in 2 – 3 volumes of ice-cold Extraction Buffer.
 - 10.2.4 Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.
 - 10.2.5 Incubate sample on ice for 15 – 30 minutes.
 - 10.2.6 Centrifuge samples for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.
 - 10.2.7 Collect supernatant and transfer to a clean tube.
 - 10.2.8 Keep on ice.
 - 10.2.9 Measure protein concentration and adjust to 25 – 200 µg per 50 µL with Extraction Buffer.
- 10.3 **Plasma, Serum and Urine and other biological fluids:**
Use sample directly in the assay.

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

11. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all controls and samples in duplicate.**

11.1 **Set up Reaction wells:**

- Background well = 50 μ L Extraction Buffer.
- Sample wells = 1 - 50 μ L samples (adjust volume to 50 μ L/well with Extraction Buffer).

NOTE: Background reading from substrate can be quite high due to the nature of such fluorescence quenching assay.

- **Positive control well** = 50 μ L Extraction Buffer + 4 μ L reconstituted active β -Secretase.
- **Negative control well** = 50 μ L Extraction Buffer + 4 μ L reconstituted active β -Secretase + 2 μ L β -Secretase Inhibitor.
- (Optional) Negative sample control well = 1 – 50 μ L samples + 2 μ L β -Secretase Inhibitor.

11.2 Add 50 μ L of Reaction Buffer/2X Reaction Buffer to each well (including background and controls).

11.3 If using Inhibitor, gently mix, then pre-incubate 5 – 10 minutes at 37°C BEFORE adding substrate.

11.4 Add 2 μ L β -Secretase substrate to all wells.

11.5 Cover plate, tap gently to mix.

11.6 Incubate at 37°C for 30 minutes in the dark.

11.7 Measure output on a fluorescent microplate reader at Ex/Em = 335/495 nm.

12. CALCULATIONS

- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
 - 12.1 Average the duplicate reading for each sample.
 - 12.2 The reading from the Sample Background Control well should be subtracted from all samples (treated and untreated) before calculating the fold increase in secretase activity.

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

13. TYPICAL DATA

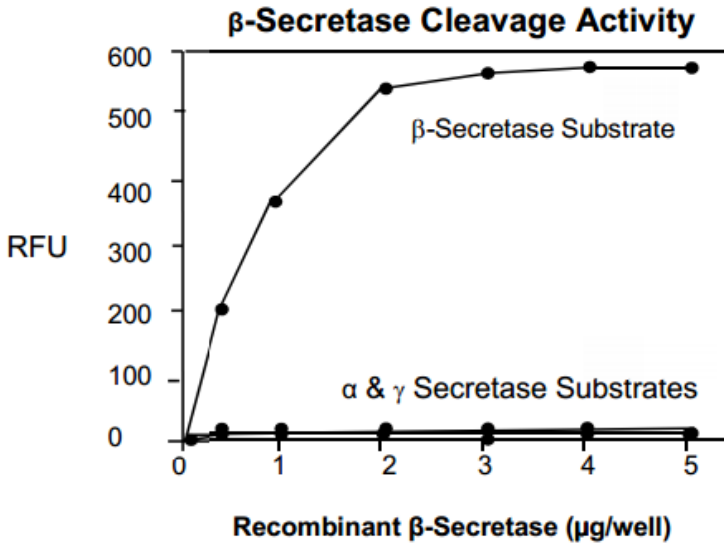


Figure 1. Typical β-Secretase cleavage activity that exclusively cleaves β-Secretase using fluorometric reading.

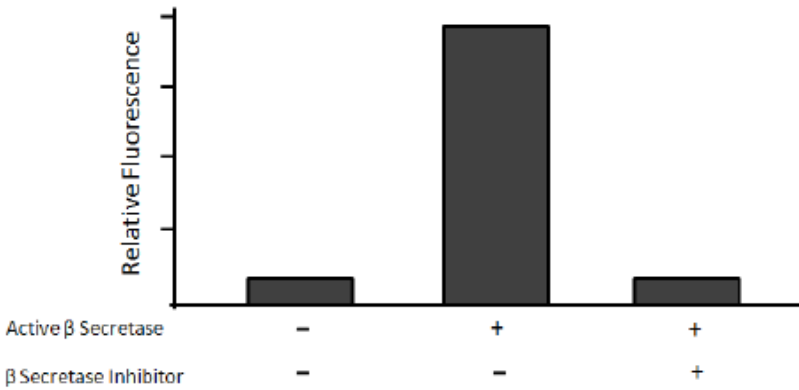


Figure 2: Effect of β-Secretase inhibitor on the active β-Secretase provided in kit ab65357.

14. QUICK ASSAY PROCEDURE

NOTE: *This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare buffers, enzyme, inhibitor and substrate (aliquot if necessary); get equipment ready.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for treated and untreated samples (50 μ L), background control wells (50 μ L), positive and negative controls (50 μ L).
- Add 2 μ L of active β -Secretase to positive control well only; add 2 μ L β -Secretase Inhibitor to negative control well only.
- Add 50 μ L of Reaction Buffer/2X Reaction Buffer to each well (including background and controls).
- If using Inhibitor, gently mix and pre-incubate 5-10 mins at 37°C BEFORE adding substrate.
- Add 2 μ L β -Secretase substrate. DO not add substrate to Background control sample well.
- Incubate plate at 37°C for 1 hour in the dark.
- Measure plate in a fluorescent reader at Ex/Em= 335/495 nm.

RESOURCES

15. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

RESOURCES

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 μL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

16. FAQ

We collected about $5-6 \times 10^6$ cells, added 100 μL extraction buffer, but after incubating the cell lysate on ice for 10 minutes and centrifuge at $10,000 \times g$ for 5 minutes there seemed to be too much protein to collect the supernatant. Were too many cells used? Do you have any other suggestions?

Two suggestions: (1). When collecting cells, make sure you remove as much cell medium as possible. Spin a second time if necessary. This will allow the extraction buffer to lyse the cells more efficiently. (2). Since cell size are quite different, you may increase the volume of extraction buffer to 200 μL , so the extraction will be more complete.

Which BACE enzymes are assayed in this kit?

BACE-1 (beta-site APP Cleaving Enzyme 1) and BACE 2 are both aspartic proteases. Our kit does not distinguish between the two isoforms. BACE1 is the primary protease responsible for APP cleavage and is better studied. It is possible that both enzymes are assayed by this kit.

Can the incubation period be reduced from 1 hour?

A 1 hour incubation period is ideal for the reaction, but 30 minutes to 1 hour should not make a big difference. However, there may be a slight decrease in signal.

What reagents should be added to the Background Control well?

Extraction Buffer, reaction buffer and substrate are required for the Background control well. There is no source of β -secretase enzyme. The substrate has 2 reporter molecules and can quench and release fluorescence and this background fluorescence without enzyme activity needs to be subtracted from all readings.

17. INTERFERENCES

These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure

- RIPA: contains SDS which can destroy/decrease the activity of the enzyme.
- Protease inhibitors.

18. NOTES

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RESOURCES



Technical Support

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