

## ab282921 – Beta-Secretase (BACE1) Activity Assay Kit (Fluorometric)

For the measurement of BACE1 activity in various tissues/cells.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab282921>

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.

**Storage and Stability:** Entire assay kit should be stored at -80°C, protected from light.

### Materials Supplied

Item	Quantity	Storage Condition
β-Secretase Extraction Buffer/BACE1 Extraction Buffer	25 mL	-20°C
β-Secretase Reaction Buffer/BACE1 Assay Buffer	10 mL	-20°C
β-Secretase Substrate/BACE1 Substrate (in DMSO)	200 µl	-20°C
Active β-Secretase/BACE1 Positive Control	20 µl	-80°C
EDANS Standard (100 µM)	100 µl	-20°C
β-Secretase Inhibitor Control/BACE1 Inhibitor Control (in DMSO)	100 µl	-20°C

### Materials Required, Not Supplied

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

- 96-well black plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

**Reagent Preparation** Before using the kit, spin the tubes prior to opening.

**β-Secretase Extraction Buffer/BACE1 Extraction Buffer and β-Secretase Reaction Buffer/BACE1 Assay Buffer:**

Warm to room temperature (RT) before use. Store at 4°C.

**β-Secretase Substrate/BACE1 Substrate, EDANS Standard and β-Secretase Inhibitor Control/BACE1 Inhibitor Control:** Thaw to RT. Ready to use. Store at -20°C. Use within two months.

**Active β-Secretase/BACE1 Positive Control:** Keep on ice while in use. Aliquot and store immediately at -80°C. Use within two months.

### Assay Protocol

#### Sample Preparation

##### Cells or tissue lysate

1. Rapidly homogenize tissue (10 mg) or cells (10 x 10<sup>6</sup>) with 500 µl ice-cold β-Secretase Extraction Buffer/BACE1 Extraction Buffer, and place on ice for 10 min.
2. Centrifuge at 10,000 x g for 5 min at 4°C and collect the supernatant.
3. Use saturated ammonium sulfate to precipitate proteins and remove interferences such as small molecules: Aliquot homogenized samples (100 µl) to a clean centrifuge tube, add saturated 4.32 M ammonium sulfate bringing saturation to 65% (1 volume of sample

+ 2 volumes of 4.32 M ammonium sulfate).

4. Place on ice for 30 mins. Spin down samples at 10,000 x g at 4°C for 10 min, discard supernatant, and resuspend the pellet back to the original volume with β-Secretase Reaction Buffer/BACE1 Assay Buffer.
5. Add 2-50 µl Samples into a 96 well black plate. Adjust final volume to 50 µl with β-Secretase Reaction Buffer/BACE1 Assay Buffer.
6. Active β-Secretase/BACE1 Positive Control: Add 2-5 µl of Active β-Secretase/BACE1 Positive Control into the wells and adjust final volume to 50 µl with β-Secretase Reaction Buffer/ BACE1 Assay Buffer.

#### Notes:

- For Unknown Samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.
- For Unknown Samples or Samples exhibiting high background, prepare parallel Sample well(s) as Sample Background Controls

### EDANS Standard Curve:

1. Dilute EDANS Standard to 10 µM by adding 10 µl of 100 µM EDANS to 90 µl β-Secretase Reaction Buffer/BACE1 Assay Buffer.
2. Add 0, 2, 4, 6, 8 and 10 µl of diluted 10 µM EDANS Standard into a series of wells to generate 0, 20, 40, 60, 80 and 100 pmol/well of EDANS Standard.
3. Adjust volume to 100 µl/well with β-Secretase Reaction Buffer/BACE1 Assay Buffer.

### Reaction Mix Preparation

Mix enough reagents for the number of assays to be performed. For each well, prepare a total of 50 µl Mix containing:

Item	Reaction Mix	Background Mix*
β-Secretase Reaction Buffer/BACE1 Assay Buffer	48 µl	47 µl
β-Secretase Substrate/BACE1 Substrate (in DMSO)	2 µl	2 µl
β-Secretase Inhibitor Control/BACE1 Inhibitor Control (in DMSO)	----	1 µl

Add 50 µl of the Reaction Mix to each well containing Test Samples and Active β-Secretase/BACE1 Positive Control well(s).

\* For Unknown Samples or Samples with high background, add 50 µl of Background Control Mix in a duplicate well(s).

### Measurement

Measure the plate immediately at Ex/Em= 345/500 nm in a kinetic mode for 10-60 min at 37°C. **Note:** Incubation time depends on the BACE1 activity in the Samples. We recommend measuring fluorescence in kinetic mode, and choosing two time points (t<sub>1</sub> & t<sub>2</sub>) in the linear range to calculate the BACE1 activity of the Samples. If low activity is expected, longer incubation times may be needed.

The EDANS Standard Curve can be read in Endpoint mode (i.e. at the end of incubation time).

## Calculation

1. Subtract the 0 Standard reading from all Standard readings.
2. Plot the EDANS Standard Curve.
3. Correct Sample Background by subtracting the value derived from the Background Control from all Sample readings if necessary.
4. Calculate the BACE1 activity of the Test Sample:  $\Delta\text{RFU} = \text{RFU}_2 - \text{RFU}_1$ . Apply the  $\Delta\text{RFU}$  to the EDANS Standard Curve to get B pmol of EDANS generated by BACE1 during the reaction time ( $\Delta t = t_2 - t_1$ ).

$$\text{Sample BACE1 Activity} = B / (\Delta t \times V) \times D = \text{pmol/min}/\mu\text{l} = \mu\text{U}/\mu\text{l} = \text{mU/ml}$$

### Where:

**B** is the EDANS amount from Standard Curve (pmol)

$\Delta t$  is the reaction time (min) V is the Sample volume added into the reaction well ( $\mu\text{l}$ )

**D** is the dilution factor

**Unit Definition:** One unit of EDAN is the amount of enzyme that will generate 1.0  $\mu\text{mol}$  of EDANS per min at pH 4.5 at 37°C

### Technical Support

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