

Product Information

SensiZyme BACE1 Activity Assay Kit

Catalog Number **CS1060**
 Storage Temperature $-20\text{ }^{\circ}\text{C}$

TECHNICAL BULLETIN

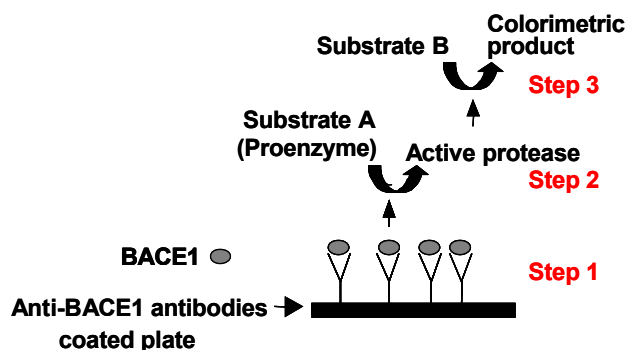
Product Description

BACE1 (β -secretase or beta-site APP-cleaving enzyme) and γ -secretase are proteases that cleave the amyloid precursor protein (APP) to produce amyloid beta peptide ($A\beta$). The accumulation of $A\beta$ in the brain is believed to be a primary cause for the progression of Alzheimer's disease. Since there is no effective drug for the treatment of Alzheimer's disease, there is an intense interest in studying the inhibition of γ - and β -secretases for therapeutic intervention in Alzheimer's patients.¹⁻⁴ Knockout studies show that BACE1 is critical for $A\beta$ generation. Transgenic mice lacking BACE1 do not produce $A\beta$, but show an otherwise normal phenotype with no detrimental effects on viability or morphology.⁵ This raises the possibility that therapeutic BACE1 inhibition could be accomplished without major toxicity.

The BACE1 Activity Assay Kit provides all the reagents required for highly sensitive detection of BACE1 activity in cell extracts, cell culture media, tissue extracts and purified enzyme preparations, and for inhibitor screening.

The protease activity measurement is based on a multistep series of reactions (see Figure 1).⁶

Figure 1.
 Principle of BACE1 Assay



Step 1: The BACE1 containing extract is applied into a well coated with a BACE1 specific antibody (supplied with the kit).

Step 2: A modified protein substrate (Substrate A) is added to the well. Substrate A is a proenzyme containing the BACE1 protease specific cleavage site fused to another protease. The proenzyme substrate is cleaved by BACE1 to form an active "new" protease.

Step 3: A colorimetric peptide substrate for the "new" protease (Substrate B) is added to the well and is cleaved by the "new" protease. The change in the absorption of the chromogenic product is measured at 405 nm. The BACE1 activity (expressed in ng/ml) is directly proportional to the generation of color.

This assay is sensitive and specific. The enhanced sensitivity is achieved by the signal amplification via the chain reaction. The specificity is achieved by both the immunochemical isolation of the BACE1 enzyme from the extract by specific antibodies bound to the 96-well plate, and the use of an enzyme substrate (Substrate A) containing a BACE1 specific cleavage site.

Components

The kit is sufficient for 96 reactions in the anti-BACE1 coated 96-well plate.

Assay Buffer Catalog Number A2481	6 ml
Wash Buffer Catalog Number W3392	80 ml
BACE1 Standard Catalog Number B5686	25 μ l
Substrate A (proenzyme) 10 \times Catalog Number S2447	500 μ l

Substrate B
Catalog Number S2322

50 μ l

DTT
Catalog Number D7059

400 μ l

Anti-BACE1 coated 96-well plate
Catalog Number B5561

1 each

Equipment Needed but Not Provided

- Spectrophotometer to read 96-well plate

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

The anti-BACE1 coated 96-well plate (Product Number B 5561) is composed of twelve 8-well strips. Before use, allow the anti-BACE1 coated 96-well plate (the frame with the required number of strips) to warm to room temperature. Unused strips should be stored in a tightly closed nylon bag with desiccant pack at 2–8 °C.

Before performing the assay, thaw the Wash Buffer (Catalog Number W3392) at room temperature and Substrate A (proenzyme) 10 \times (Catalog Number S2447) on ice. Ensure the solutions are homogenous by gentle mixing.

Substrate A Working Solution - Dilute an aliquot of Substrate A (proenzyme) 10 \times , 10-fold with Wash Buffer (Catalog Number W3392). Keep the prepared Substrate A Working Solution on ice until needed for the reaction. Store the remaining Substrate A at –20 °C and the Assay Buffer at 2–8 °C. 50 μ l of Substrate A Working Solution are sufficient for a reaction in a single well.

Standard Solutions – Just prior to beginning the assay, dilute an aliquot of the 100 μ g/ml BACE1 Standard (Catalog Number B5686) to a concentration of 200 ng/ml by diluting 2 μ l of the BACE1 Standard in 1 ml of Wash Buffer (Product Number W3392). Mix well by vortexing. Prepare serial dilutions according to Table 1. Mix well after each dilution. Store the Standard Solutions on ice until use. The Standard Solutions will be used to determine a Standard Curve of BACE1 activity.

Table 1.

Serial Dilutions of 200 ng/ml BACE1 Standard Solution

Standard sample	BACE1 standard (μ l)	Wash buffer (μ l)	BACE1 Standard, final concentration (ng/ml)
1	25 (from 200 ng/ml)	475	10
2	250 (from 10 ng/ml)	250	5
3	250 (from 5 ng/ml)	250	2.5
4	250 (from 2.5 ng/ml)	250	1.25
5	250 (from 1.25 ng/ml)	250	0.625
6	250 (from 0.625 ng/ml)	250	0.312
7	250 (from 0.312 ng/ml)	250	0.156
8	250 (from 0.156 ng/ml)	250	0.078
9 blank	0	250	0

Note: For detection of samples with high BACE1 activity, use a standard curve consisting of standard samples 1-5 and the blank. For detection of samples with low BACE1 activity, use a standard curve consisting of standard samples 3-8 and the blank.

After the overnight incubation (Procedure, step 6) thaw Substrate B (Product Number S2322) and the Assay Buffer (Product Number A2481) at room temperature. Thaw the DTT on ice. Ensure the solutions are homogenous by gentle mixing.

Storage/Stability

The kit ships on dry ice and storage at –20 °C is recommended for all the components **except** the Anti-BACE1 coated 96-well plate that should be stored at 2–8 °C. Once thawed, the Assay Buffer and Wash Buffer should be stored at 2–8 °C.

Procedure

BACE Activity Assay

For each assay, use coated wells and Substrate A only from the same lot.

It is recommended to work in duplicates.

1. Pipette 100 μ l of each standard sample and blank (see Table 1) separately into the appropriate wells.
2. Pipette 100 μ l of the test samples into other wells.
Note: Samples with a high BACE1 activity should be diluted with Wash Buffer.
3. Cover the plate with the lid and incubate for 2 hours at 2–8 °C.
4. Aspirate the solution from the plate wells and wash the wells 4 times with 100 μ l of Wash Buffer each time.
5. Blot the plate on tissue paper to remove any residual solution.
6. Pipette 50 μ l of Substrate A Working Solution into each well. Cover the plate with the lid and incubate overnight at room temperature in a humidified chamber (i.e., a closed box with a source of humidity such as wet paper).
7. At the end of the incubation, prepare a Reagent Mixture of Assay Buffer with Substrate B and DTT (see Table 2). 50 μ l of the Reagent Mixture are required for a single well.

Table 2.

Preparation of Reaction Mixture for 10 Wells

Assay buffer	486 μ l
Substrate B	4 μ l
DTT	10 μ l

Mix well by vortexing.

8. Add 50 μ l of the Reagent Mixture to each well. Cover the plate with the lid and shake for 20 seconds.
9. Incubate the plate at room temperature for several hours. The incubation time depends on the BACE1 activity in the sample:
 - for BACE1 concentrations in the range of 0.6–10 ng/ml incubate the plate for 1 hour
 - for BACE1 concentrations in the range of 0.08–2.5 ng/ml incubate for 3 hours or longer.
10. Remove the lid and measure the absorbance at 405 nm (A_{405}) using a plate reader after appropriate incubation (step 9). If the signal is low, continue the incubation for additional 1–2 hours to increase the assay sensitivity.

Calculations

1. Calculate the average absorbance (of the duplicates) of the blank, of each standard concentration, and of the test samples. Subtract the average blank absorbance value from the average value of each standard and sample.
2. Plot the average absorbance of each standard concentration (y-axis) as a function of BACE1 concentration in the well (x-axis).

Figures 1 and 2 show representative Standard Curves of BACE1 activity at high or low concentrations of BACE1, respectively. The difference in sensitivity is achieved by longer incubation periods.

Figure 2.

Standard Curve for BACE1 (0.6–10 ng/ml)

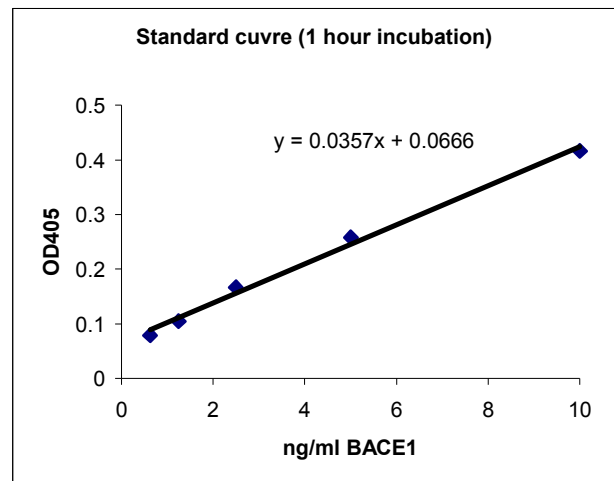
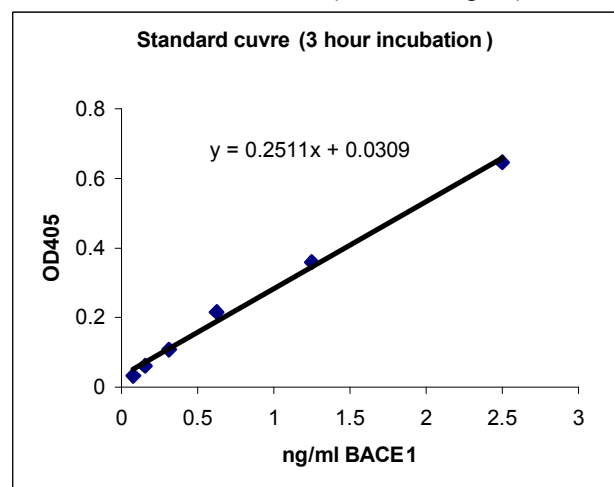


Figure 3.

Standard Curve for BACE1 (0.08–2.5 ng/ml)



References

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3. Rochette, M.J., and Murphy, M.P., γ -secretase: substrates and inhibitors. *Mol. Neurobiol.*, **26**, 81-95 (2002).
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6. Verheijen, J.H., *et al.*, Detection of a soluble form of BACE-1 in human cerebrospinal fluid by a sensitive activity assay. *Clin. Chem.*, **52**, 1168-1174 (2006).

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United States Patent 5,811,252
European Patent 0,691,409B1
Japanese Patent Application 8,056,665A2

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