Code No. 27752

BACE1 Assay Kit - IBL

INTRODUCTION

Neuropathologically Alzheimer's disease (AD) is characterized by widespread deposition of amyloid β protein (Aβ) in the cerebral cortex, and since the intracerebral accumulation of Aβ is highly specific for AD and the gene mutation responsible for familial amyloidosis causes Aβ to increase, Aβ is thought to be of extremely great significance in the pathogenetic mechanism of AD. Aβ is generated by proteolysis of the Aβ precursor protein (APP), a type I membrane protein. The enzyme that cleaves it at the N-terminal is called β secretase, and it is regarded as being the protease that acts at the initial step in the production of Aβ. The major β secretase has been identified as β-site APP Cleaving Enzyme 1 (BACE1), and BACE2 has been isolated as its homologue.

PRINCIPLE

This kit is a solid phase sandwich ELISA using 2 kinds of high specific antibodies. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The strength of coloring is in proportional to the quantities of BACE1.

MEASUREMENT RANGE

1.56 - 100 ng/mL

INTENDED USE

For research use only, not for use in diagnostic procedures.

■ It is used to measure BACE1 in brain extracts or cultured cell lysate.
(Actual example 1)
Preparation of the brain extract solution
Add 5 volumes of extraction buffer (1% CHAPS in TBS pH7.6) to the brain sample, and homogenize. When homogenization is complete, let stand on ice for at least 3 hours, centrifuge at 70,000 rpm for 20 minutes at 4°C, and then appropriately dilute the supernatant with "4, EIA buffer" in the kit, and use for measurement.
(Actual example 2)
Preparation of the cultured cell Lysate
Add 0.5mL Lysis Buffer (10mM Tris (pH7.4), 0.15M NaCl, 3% TritonX-100, 0.3mM PMSF) to the cells pellet ($5x10^6$- $1x10^7$ indication), and sonicate for 3 times each 20 seconds. Centrifuge at 15,000 rpm for 15 minutes, and use the supernatant for measurement.

■ This product is capable of measuring each of human, mouse, rat, recombinant and native BACE1.

KIT COMPONENT

1	Precoated plate	: Anti-BACE1 (N42) Rabbit IgG Affinity Purify	96Well x 1
2	Labeled antibody Conc.	: (30X) HRP conjugated Anti-BACE1 (C) Rabbit IgG Fab' Affinity Purify	0.4mL x 1
3	Standard	: Human BACE1 COS-7 Lysate	0.5mL x 2
4	EIA buffer		30mL x 1
5	Solution for Labeled antibody	: 1% BSA, 0.05%Tween 20 in PBS	12mL x 1
6	Chromogen	: TMB solution	15mL x 1
7	Stop solution	: 1N H_2SO_4	12mL x 1
8	Wash buffer Conc.	: (40X) 0.05% Tween20 in phosphate buffer	50mL x 1

OPERATION MANUAL

1. Materials needed but not supplied

- Plate reader (450nm)
- Graduated cylinder and beaker
- Refrigerator (as 4°C)
- Paper towel
- Incubator (37℃±1°C)
- Washing bottle for precoated plate
- Disposable test tube for "2, Labeled antibody Conc." and "6, Chromogen"
- Micropipette and tip
- Deionized water
- Graph paper (log/log)
- Tube for dilution of Standard

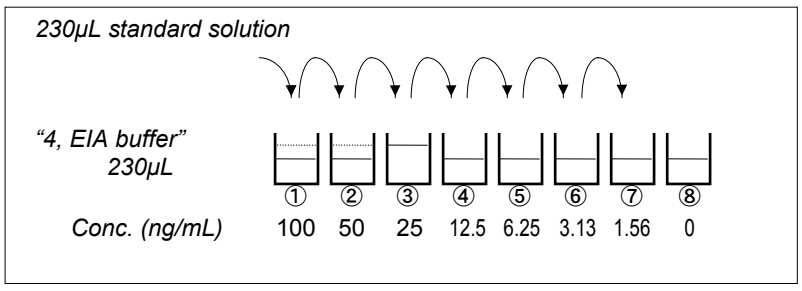
2. Preparation

1) Preparation of wash buffer
"8, Wash buffer Conc." is a concentrated (40X) buffer. The temperature of "8, Wash buffer Conc." shall be adjusted to room temperature and then, mix it gently and completely before use. Dilute 50mL of "8, Wash buffer Conc." with 1,950 mL of deionized water and mix it. This is the wash buffer for use. This prepared wash buffer shall be stored in refrigerator and used within 2 weeks after dilution.
2) Preparation of Labeled antibody
"2, Labeled antibody Conc." is a concentrated (30X). Dilute "2, Labeled antibody Conc." with "5, Solution for Labeled antibody" in 30 times according to required quantity into a disposable test tube. Use this resulting solution as Labeled antibody.
Example)
In case you use one slit (8 well), the required quantity of Labeled antibody is 800μL. (Dilute 30μL of "2, Labeled antibody Conc." with 870μL of "5, Solution for Labeled antibody" and mix it. And use the resulting solution by 100μL in each well.)
This operation should be done just before the application of Labeled antibody.
The remaining "2, Labeled antibody Conc." should be stored at 4°C in firmly sealed vial.
3) Preparation of Standard
Put just 0.5 mL of "4, EIA buffer" into the vial of "3, Standard" and mix it gently and completely. This solution is 200 ng/mL BACE1 standard.
4) Dilution of Standard
Prepare 8 tubes for dilution of "3, Standard". Put 230μL each of "4, EIA buffer" into the tube.
Specify the following concentration of each tube.

Tube-1	100 ng/mL
Tube-2	50 ng/mL
Tube-3	25 ng/mL
Tube-4	12.5 ng/mL
Tube-5	6.25 ng/mL
Tube-6	3.13 ng/mL
Tube-7	1.56 ng/mL
Tube-8	0 ng/mL (Test Sample Blank)

Put 230μL of Standard solution into tube–1 and mix it gently. Then, put 230μL of tube-1 mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 100 ng/mL and 1.56 ng/mL. Tube-8 is the test sample blank as 0 ng/mL.

See following picture.



5) Dilution of test sample
Test sample should be diluted with "4, EIA buffer" accordingly.
If the concentration of BACE1 in samples may not be estimated in advance, the pre-assay with several different dilutions will be recommended to determine the proper dilution of samples.

3. Measurement procedure

All reagents shall be brought to room temperature approximately 30 minutes before use. Then mix it gently and completely before use. Confirm no change in quality of the reagents. Standard curve shall be prepared simultaneously with the measurement of test samples.

Reagents	Test Sample	Standard	Test Sample Blank	Reagent Blank
	Test sample 100 μL	Diluted standard (Tube 1~7) 100 μL	EIA buffer (Tube-8) 100 μL	EIA buffer 100 μL
Incubation for 60 minutes at 37°C with plate lid				
Washing 7 times				
Labeled Antibody	100 μL	100 μL	100 μL	-
Incubation for 30 minutes at 4°C with plate lid				
Washing 9 times				
Chromogen	100 μL	100 μL	100 μL	100 μL
Incubation for 30 minutes at room temperature (shielded)				
Stop solution	100 μL	100 μL	100 μL	100 μL
Read the plate at 450nm against a Reagent blank within 30 minutes after addition of Stop solution.				

1) Determine wells for reagent blank. Put 100 μL each of "4, EIA buffer" into the wells.
2) Determine wells for test sample blank, test sample and diluted standard. Then, put 100 μL each of test sample blank (tube-8), test sample and dilutions of standard (tube-1-7) into the appropriate wells.
3) Incubate the precoated plate for 60 minutes at 37°C after covering it with plate lid.
4) Wash each well of the precoated plate vigorously with wash buffer using washing bottle. Then, fill each well with wash buffer and leave the precoated plate lay for 15-30 seconds. Remove wash buffer completely from the precoated plate by snapping. This procedure must be repeated more than 7 times. Then, remove the remaining liquid from all wells completely by snapping the precoated plate onto paper towel.
In case of using a plate washer, after 4 times washing with plate washer, washing with above washing bottle must be repeated 3 times.
5) Pipette 100μL of labeled antibody solution into the wells of test samples, diluted standard and test sample blank.
6) Incubate the precoated plate for 30 minutes at 4°C after covering it with plate lid.
7) Wash the precoated plate 9 times in the same manner as 4).
8) "6, Chromogen" should be taken the required quantity into a disposable test tube. Then, pipette 100 μL from the test tube into the wells. Please do not return the rest of the test tube to "6, Chromogen" bottle due to avoid to cause of contamination.
9) Incubate the precoated plate for 30 minutes at room temperature in the dark. The liquid will turn blue by the addition of "6, Chromogen".
10) Pipette 100 μL of "7, Stop solution" into the wells. Mix the liquid by tapping the side of precoated plate. The liquid will turn yellow by the addition of "7, Stop solution".
11) Remove any dirt or drop of water on the bottom of the precoated plate and confirm there is no bubble on the surface of the liquid. Then, run the plate reader and conduct measurement at 450 nm against a reagent buffer.
The measurement shall be done within 30minutes after the addition of "7, Stop solution".

SPECIAL ATTENTION

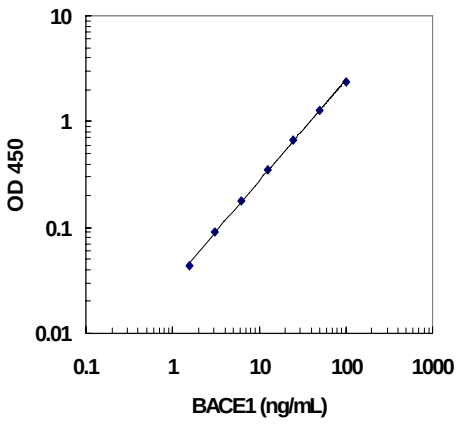
1) Test samples should be measured soon after collection. For the storage of test samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at low temperature and mix them completely before measurement.
2) Test samples should be diluted with “4, EIA buffer”, if the need arises.
3) Duplicate measurement of test samples and standard is recommended.
4) Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
5) Use only wash buffer contained in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.
6) Remove the wash buffer completely by tapping the precoated plate on paper towel.
   Do not wipe wells with paper towel.
7) “6, Chromogen” should be stored in the dark due to its sensitivity against light. “6, Chromogen” should be avoided contact with metals.
8) Measurement should be done within 30 minutes after addition of “7, Stop solution”.

CALCULATION OF TEST RESULT

Subtract the absorbance of test sample blank from all data, including standards and unknown samples before plotting. Plot the subtracted absorbance of the standards against the standard concentration on log-log graph paper. Draw the best smooth curve through these points to construct the standard curve. Read the concentration for unknown samples from the standard curve.

Example of standard curve

Conc. (ng/mL)	Absorbance (450nm)
100	2.387
50	1.319
25	0.694
12.5	0.378
6.25	0.202
3.13	0.113
1.56	0.067
0 (Test Sample Blank)	0.023



＊ The typical standard curve is shown above. This curve can not be used to derive test results. Please run a standard curve for each assay.

PERFORMANCE CHARACTERISTICS

1. Titer Assay (Samples with standard added are used.)

Specimen	Titer (X)	Measurement Value (ng/mL)	Theoretical Value (ng/mL)	%
Rat brain extracts (SD)	20	50.52	63.61	79.4
	40	29.85	34.05	87.7
	80	15.29	17.02	89.8
Mouse brain extracts (C57BL/6)	20	14.83	16.42	90.3
	40	7.95	8.46	93.9
	80	3.69	4.19	88.0
10%FCS added RPMI-1640	2	22.46	25.00	89.8
	4	10.82	12.50	86.6
	8	5.24	6.25	83.8

2. Added Recovery Assay

Specimen	Theoretical Value (ng/mL)	Measurement Value (ng/mL)	%
Rat brain extracts (SD) (x40)	47.27	43.58	92.2
	34.77	34.34	98.7
	28.52	29.54	103.5
Mouse brain extracts (C57BL/6) (x20)	15.35	13.25	86.3
	9.10	7.93	87.1
	5.98	5.78	96.7
10%FCS added RPMI-1640 (x2)	50.00	46.43	92.9
	25.00	23.03	92.1
	12.50	12.24	97.9

3. Intra - Assay

Measurement Value (ng/mL)	SD value	CV value (%)	n
49.37	1.45	2.9	24
11.62	0.35	3.0	24
2.77	0.15	5.4	24

4. Inter - Assay

Measurement Value (ng/mL)	SD value	CV value (%)	n
47.84	2.53	5.3	38
11.23	0.67	6.0	38
2.64	0.22	8.3	38

5. Specificity

Compound	Cross Reactivity
Human BACE1	100.0 %
Human BACE1 (1-460)	< 0.1%

6. Sensitivity

0.16 ng/mL
The sensitivity for this kit was determined using the guidelines under the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols. (National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.)

PRECAUTION FOR INTENDED USE AND/OR HANDLING

1. All reagents should be stored at 2 - 8°C. All reagents shall be brought to room temperature approximately 30 minutes before use.
2. “3, Standard” is lyophilized products. Be careful to open this vial.
3. “7, Stop solution” is a strong acid substance. Therefore, be careful not to have your skin and clothes contact “7, Stop solution” and pay attention to the disposal of “7, Stop solution”.
4. Dispose used materials after rinsing them with large quantity of water.
5. Precipitation may occur in “2, Labeled antibody Conc.”, however, there is no problem in the performance.
6. Wash hands after handling reagents.
7. Do not mix the reagents with the reagents from a different lot or different kit.
8. Do not use the expired reagents.
9. This kit is for research purpose only. Do not use for clinical diagnosis.

STORAGE AND THE TERM OF VALIDITY

Storage Condition : 2 - 8 °C
The expiry date is specified on outer box.

REFERENCE

1. Kitazume-Kawaguchi S, Dohmae N, Takio K, Tsuji S, Colley KJ. The relationship between ST6Gal I Golgi retention and its cleavage-secretion. : Glycobiology. 1999 Dec;9(12):1397-406.
2. Kitazume S, Tachida Y, Oka R, Shirotani K , Saido TC , Hashimoto Y. Alzheimer's beta-secretase, beta-site amyloid precursor protein-cleaving enzyme, is responsible for cleavage secretion of a Golgi-resident sialyltransferase. : Proc Natl Acad Sci U S A. 2001 Nov 20;98(24):13554-9.
3. Kitazume S, Tachida Y, Oka R, Kotani N, Ogawa K, Suzuki M, Dohmae N, Takio N, Saido TC, Hashimoto Y. Characterization of alpha 2,6-Sialyltransferase Cleavage by Alzheimer's beta -Secretase (BACE1). : J Biol Chem. 2003 Apr 25;278(17):14865-71.

Version 1.2

Made in Japan