Code No. 27716

Human Amyloidβ (N3pE-42) Assay Kit - IBL

INTRODUCTION

The first case of Alzheimer's disease was defined and reported in 1907 by the German scientist, Dr. A. Alzheimer. His studies have shown that this is the main cause of dementia in the elderly. The plaques which appear in the brains of Alzheimer's disease patients are mostly constituted by the Amyloid β protein (Aβ). Aβ is a peptide which consists of 40 or 42 (43) amino acids, and reports show that this is cleaved by β- and γ- secretase from the amyloid precursor protein. APP is a trans-membrane protein consisting of 695, 751, or 770 amino acids.
Reports have shown many variants of Aβ exist and are clarified into the culture supernatant from the APP cDNA transfected mouse neuroblastoma cell.
Furthermore, in 1995, a dominant and differential deposition of distinct β amyloid peptide species, Aβ (N3pE), in senile plaques was found by Saido *et al*. This modified molecule, starting at the 3rd amino terminal residue, glutamate, was discovered to convert to pyroglutamate through intramolecular dehydration.
This kit can be measured Human Aβ (N3pE-42). For measuring Human Aβ (N3pE-40), use IBL Code No.27418, Human Amyloid β (N3pE-40) Assay Kit .
When measuring Aβ (1-40) variants cleaved N terminal side by any cause, please use IBL Code No.27713, Human Amyloid β (1-40) Assay Kit. The IBL Code No.27714, Human Amyloid β (1-40) (N) Assay Kit and IBL Code No.27718 Human Amyloid β (1-40) (FL) Assay Kit are separately prepared when measuring Aβ (1-40) which held N terminal side completely. In addition, Human Amyloid β (1-40) (N) Assay Kit uses a polyclonal antibody as Labeled antibody. On the other hand, the Human Amyloid β (1-40) (FL) Assay Kit uses a monoclonal antibody.
Moreover, in assay of Aβ (1-42) variants cleaved N terminal side, it is a Human Amyloid β (1-42) Assay Kit similarly. When measuring Aβ (1-42) variants cleaved at N terminal side, please use IBL Code No.27711, Human Amyloid β (1-42) Assay Kit .
And, when measuring Aβ (1-42) which held N terminal side completely, please use IBL Code No.27712, Human Amyloid β (1-42) (N) Assay Kit.
Thus, it will be very useful for Alzheimer's research to assay Aβ (1-40), Aβ (1-42), Aβ (N3pE-40) and Aβ (N3pE-42), respectively.
Meanwhile, you can use IBL Code No.27729, Human Amyloid β (1-x) Assay Kit to measure Aβ variants such as Aβ (1-38), Aβ (1-40), Aβ (1-42) and Aβ (1-43) all at once.

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 proportional to the quantities of human Aβ (N3pE-42).

MEASUREMENT RANGE

7.75 - 496 pg/mL
(1.8 - 115 pmol/L, as molecular weight of Aβ (N3pE-42) is 4,310)

INTENDED USE

- Both recombinant and native forms of human Aβ (N3pE-42) can be detected with the kit.
- Human Aβ (N3pE-42) in brain tissue extract can be measured with the kit.
- Serum or plasma may become below detection sensitivity in this kit due to very few concentration of Aβ (N3pE-42) in these samples.

KIT COMPONENT

1	Precoated plate : Anti- Human Aβ (38-42) Rabbit IgG Affinity Purify	96Well x 1
2	Labeled antibody Conc. : (30X) HRP conjugated Anti- Human Aβ N3pE (8E1) Mouse IgG Fab'Affinity Purify	0.4mL x 1
3	Standard : Human Aβ (N3pE-42)	0.5mL x 2
4	EIA buffer*	30mL x 1
5	Solution for Labeled antibody*	12mL x 1
6	Chromogen : TMB solution	15mL x 1
7	Stop solution*	12mL x 1
8	Wash buffer Conc.*	50mL x 1

OPERATION MANUAL

1. Materials needed but not supplied

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

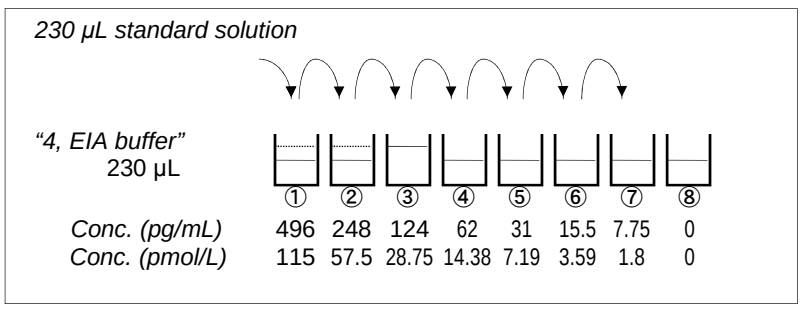
2. Preparation

1) Preparation of wash buffer
"8, Wash buffer Conc." is a concentrated (40X) buffer. Adjust the temperature of "8, Washing buffer Conc." to room temperature and then, mix it gently and completely before use. Dilute 50 mL 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 strip (8 well), the required quantity of Labeled antibody is 800 µL. (Dilute 30 µL of "2, Labeled antibody Conc." with 870 µL of "5, Solution for Labeled antibody" and mix it. And use the resulting solution by 100 µL in each well.)
<u>This operation should be done just before the application of Labeled antibody.</u>
The remaining "2, Labeled antibody Conc." should be stored at 4℃ in firmly sealed vial.
3) Preparation of Standard
Put just <u>0.5 mL</u> of deionized water into the vial of "3, Standard" and mix it gently and completely. This solution is 992 pg/mL Human Aβ (N3pE-42) standard. The standards enclosed in this kit can be frozen and stored after reconstitution. However the freeze-thaw shall not be repeated.
4) Dilution of Standard
Prepare 8 tubes for dilution of "3, Standard". Put 230 µL each of "4, EIA buffer" into the tube.
Specify the following concentration of each tube."

Tube-1	496 pg/mL
Tube-2	248 pg/mL
Tube-3	124 pg/mL
Tube-4	62 pg/mL
Tube-5	31 pg/mL
Tube-6	15.5 pg/mL
Tube-7	7.75 pg/mL
Tube-8	0 pg/mL (Test Sample Blank)

Put 230 µL of Standard solution into tube-1 and mix it gently. Then, put 230 µL of tube-1 mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 496 pg/mL and 7.75 pg/mL. Tube-8 is the test sample blank as 0 pg/mL.

See following picture.



5) Dilution of test sample
Test sample may be diluted with "4, EIA buffer" as necessary.
If the concentration of human Aβ (N3pE-42) 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. Make sure of 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 overnight at 4°C with plate lid				
4 times (wash buffer more than 350 µL)				
Labeled Antibody	100 µL	100 µL	100 µL	-
Incubation for 60 minutes at 4°C with plate lid				
5 times (wash buffer more than 350 µL)				
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 overnight at 4°C after covering it with plate lid.
4) Washing
Wash the plate with the prepared wash buffer and remove all liquid.
5) Pipette 100 µL of labeled antibody solution into the wells of test samples, diluted standard and test sample blank.
6) Incubate the precoated plate for 60 minutes at 4°C after covering it with plate lid.
7) Washing
Wash the plate with the prepared wash buffer and remove all liquid.
8) Take the required quantity of "6, Chromogen" 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 to avoid contamination.
9) Incubate the precoated plate for 30 minutes at room temperature in the dark. The liquid will turn blue by 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 addition of "7, Stop solution".

Immuno-Biological Laboratories Co., Ltd.
TEL: +81 (0)274-22-2889 FAX : +81 (0)274-23-6055
1091-1 Naka, Fujioka-Shi, Gunma, 375-0005, JAPAN
URL: http://www.ibl-japan.co.jp ***E-mail: do-ibl@ibl-japan.co.jp***

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 blank.
The measurement shall be done within 30 minutes after addition of “7, Stop solution”.

SPECIAL ATTENTION

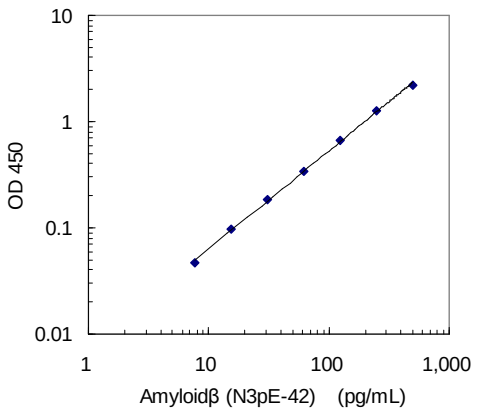
1) Test samples should be measured soon after collection. For the storage of test samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before measurement.
2) Test samples should be diluted with “4, EIA buffer”, if the need 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. (pg/mL)	Absorbance (450nm)
496	2.271
248	1.302
124	0.709
62	0.394
31	0.232
15.5	0.145
7.75	0.096
0 (Test Sample Blank)	0.049



* 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 (pg/mL)	Theoretical Value (pg/mL)	%
10%FCS added RPMI-1640	4	53.61	62.00	86.5
	8	29.51	31.00	95.2
	16	13.78	15.50	88.9
	32	7.28	7.75	93.9

2. Added Recovery Assay

Specimen	Theoretical Value (pg/mL)	Measurement Value (pg/mL)	%
10%FCS added RPMI-1640 (x8)	127.77	131.25	102.7
	65.77	67.18	102.1
	34.77	33.10	95.2

3. Intra - Assay

Measurement Value (pg/mL)	SD value	CV value (%)	n
308.69	15.90	5.2	21
75.63	3.17	4.2	21
15.83	1.03	6.5	21

4. Inter - Assay

Measurement Value (pg/mL)	SD value	CV value (%)	n
302.74	20.66	6.8	7
74.74	3.59	4.8	7
16.11	0.94	5.8	7

5. Specificity

Compound	Cross Reactivity
Human Aβ (N3pE-42)	100.0%
Human Aβ (1-40)	≦0.1%
Human Aβ (1-42)	≦0.1%
Human Aβ (1-43)	≦0.1%
Human Aβ (17-40), (P3 Form)	≦0.1%
Rat/Mouse Aβ (1-40)	≦0.1%
Rat/Mouse Aβ (1-42)	≦0.1%

6. Sensitivity

1.94 pg/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 kit.
8. Do not use 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. Selkoe DJ. Normal and abnormal biology of the beta-amyloid precursor protein. Annu Rev Neurosci. 1994;17:489-517.
2. Wang R, Sweeney D, Gandy SE, Sisodia SS. The profile of soluble amyloid beta protein in cultured cell media. Detection and quantification of amyloid beta protein and variants by immunoprecipitation-mass spectrometry. J Biol Chem. 1996 Dec 13;271(50):31894-902.
3. Saido TC, Iwatsubo T, Mann DM, Shimada H, Ihara Y, Kawashima S. Dominant and differential deposition of distinct beta-amyloid peptide species, A beta N3(pE), in senile plaques. Neuron. 1995 Feb;14(2):457-66.
4. Horikoshi Y, Mori T, Maeda M, Kinoshita N, Sato K, Yamaguchi H. Abeta N-terminal-end specific antibody reduced beta-amyloid in Alzheimer-model mice. Biochem Biophys Res Commun. 2004 Dec 10;325(2):384-7.
5. Harigaya Y, Saido TC, Eckman CB, Prada CM, Shoji M, Younkin SG. Amyloid beta protein starting pyroglutamate at position 3 is a major component of the amyloid deposits in the Alzheimer's disease brain. Biochem Biophys Res Commun. 2000 Sep 24;276(2):422-7.

Version 3. September 2016 *

IBL Amyloid β Product Lines:

Code No.	Name	Volume
27711	Human Amyloidβ (1-42) Assay Kit - IBL	96 Well
27712	Human Amyloidβ (1-42) (N) Assay Kit - IBL	96 Well
27713	Human Amyloidβ (1-40) Assay Kit - IBL	96 Well
27714	Human Amyloidβ (1-40) (N) Assay Kit - IBL	96 Well
27718	Human Amyloidβ (1-40) (FL) Assay Kit - IBL	96 Well
27720	Mouse/Rat Amyloidβ (1-40) High Specific Assay Kit - IBL	96 Well
27716	**Human Amyloidβ (N3pE-42) Assay Kit - IBL**	**96 Well**
27418	Human Amyloidβ (N3pE-40) Assay Kit - IBL	96 Well
27729	Human Amyloidβ (1- x) Assay Kit - IBL	96 Well
27724	Human sAPPα Assay Kit - IBL	96 Well
27419	Mouse/Rat sAPPα Assay Kit - IBL	96 Well
27722	Human sAPPβ-Wild Type Assay Kit - IBL	96 Well
27723	Human sAPPβ-Swedish Type Assay Kit - IBL	96 Well
27776	Human APP βCTF Assay Kit - IBL	96 Well

Distributed By:
IBL-America, Inc.
8201 Central Ave NE, Suite P
Minneapolis, MN 55432, USA
info@ibl-america.com
(888) 523 1246