Code No. 27712

Human Amyloid β (1-42) (N) Assay Kit (L) - 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 from β- and γ- secretase from the amyloid precursor protein. APP is a trans-membrane protein consisting of 695, 751, or 770 amino acids (ref. 1).
Reports have shown many variants of Aβ exist and are clarified into the culture supernatant from the APP cDNA transfected mouse neuroblastoma cell (ref. 2).
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 (ref. 3).

This kit can be measured Aβ (1-42) which held N terminal side completely.
When measuring Aβ (1-42) variants cleaved N terminal side by any cause, please use IBL Code No.27711, Human Amyloid β (1-42) Assay Kit (L). Moreover, in assay of Aβ (1-40) variants cleaved N terminal side, it is a Human Amyloid β (1-40) Assay Kit (L) similarly. When measuring Aβ (1-40) variants cleaved by N terminal side, please use IBL Code No.27713, Human Amyloid β (1-40) Assay Kit (L). And, when measuring Aβ (1-40) which held N terminal side completely, please use IBL Code No.27714, Human Amyloid β (1-40) (N) Assay Kit (L) and Code No.27718 Human Amyloid β (1-40) (FL) Assay Kit (L) .
In addition, Human Amyloid β (1-40) (N) Assay Kit (L) uses a polyclonal antibody as Labeled antibody. On the other hand, the Human Amyloid β (1-40) (FL) Assay Kit (L) uses a monoclonal antibody.
Furthermore, in measurement of Aβ (N3pE-42), please use IBL Code No.27716, Human Amyloid β (N3pE) assay Kit (L).
Thus, it will be very useful for Alzheimer's research to assay Aβ (1-40), Aβ (1-42), and Aβ (N3pE-42), respectively.
Meanwhile, you can use IBL Code No.27729, Human Amyloid β (1-x) Assay Kit (L) to measure Aβ variants such as Aβ (1-38), Aβ (1-40), Aβ (1-42) and Aβ (1-43) all at once.

IBL Amyloid β Product Lines:

Code No.	Name	Volume
27711	Human Amyloid β (1-42) Assay Kit (L) - IBL	96 Well
27712	**Human Amyloid β (1-42) (N) Assay Kit (L) - IBL**	**96 Well**
27713	Human Amyloid β (1-40) Assay Kit (L) - IBL	96 Well
27714	Human Amyloid β (1-40) (N) Assay Kit (L) - IBL	96 Well
27716	Human Amyloid β (N3pE) Assay Kit (L) - IBL	96 Well
27718	Human Amyloid β (1-40) (FL) Assay Kit (L) - IBL	96 Well
27729	Human Amyloid β (1- x) Assay Kit (L) - IBL	96 Well

PRINCIPLE

This kit is a solid phase sandwich ELISA using 2 kinds of high specific antibodies.
Tetra Methyl Benzidine (TMB) is used as coloring agent (Chromogen). The strength of coloring is in proportion to the quantities of human Aβ (1-42).

MEASUREMENT RANGE

6.25 ~ 400 pg/mL (1.4 ~88.7 pmol/L, as molecular weight of Aβ (1-42) is 4510)

INTENDED USE

- The IBL's Human Amyloid β (1-42) (N) Assay Kit (L) is a complete kit for the quantitative determination of human Aβ (1-42) in cerebrospinal fluids, cell culture media or the extract from brain tissue (ref. 4).
- If FCS etc. is contained in samples of culture supernatant, Aβ (1-42)-like in FCS may be measured. We recommend you to take the negative control.
- Serum or plasma may become below detection sensitivity in this kit due to very few concentration of Aβ (1-42) in these samples.
- It seems to be existing any interference in serum.
- Both recombinant and native forms of human Aβ (1-42) can be detected with the kit.

KIT COMPONENT

1	Precoated plate	: Anti- Human Aβ (38-42) Rabbit IgG Affinity Purify	96Wellx 1
2	Labeled antibody Conc.	: HRP conjugated Ant-Human Aβ (N) Rabbit IgG Fab' Affinity Purifiy (X30)	0.4mL x 1
3	Standard	: Human Aβ (1-42)	1.0mL x 2
4	EIA buffer	: 1% BSA, 0.05% Tween 20 in PBS	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.	: 0.05% Tween20 in phosphate buffer (X40)	50mL x 1

OPERATION MANUAL

1. Materials needed but not supplied

- Plate reader (450nm)
- Micropipette and tip
- Graduated cylinder and beaker
- Distilled water
- Refrigerator(as 4℃)
- 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 (X40) 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 1,950mL of distilled 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.

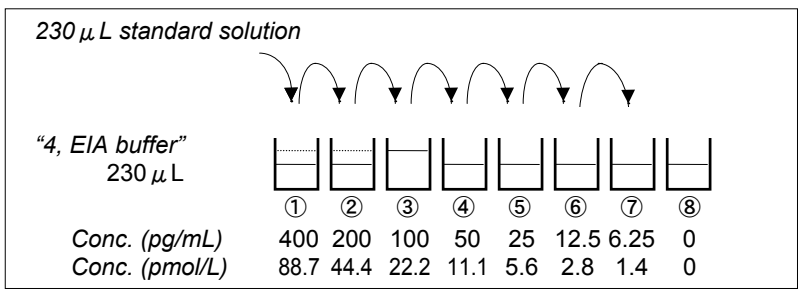
2) Preparation of Labeled antibody
"2, Labeled antibody Conc." is a concentrated (X30). Dilute "2, Labeled antibody Conc." with "5, Solution for Labeled antibody" in 30 times according to required quantity into a disposable test tube. Use this resulting solution as Labeled antibody.
Example)
In case you use one slit (8 well), the required quantity of Labeled antibody is 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.)
This operation should be done just before the application of Labeled antibody.
The remaining "2, Labeled antibody Conc." should be stored at 4℃ in firmly sealed vial.

3) Preparation of Standard
Put just 1.0 mL of distilled water into the vial of "3, Standard" and mix it gently and completely. This solution is 800 pg/mL Human Aβ (1-42) standard.

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	400 pg/mL	
Tube-2	200 pg/mL	
Tube-3	100 pg/mL	
Tube-4	50 pg/mL	
Tube-5	25 pg/mL	
Tube-6	12.5 pg/mL	
Tube-7	6.25 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 400 pg/mL and 6.25 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" if the need arises.
If the concentration of Aβ 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 overnight at 4℃ with plate lid				
Washing 7 times				
Labeled Antibody	100 μL	100 μL	100 μL	-
Incubation for 1 hour at 4℃ 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 within 30 minutes after application 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 overnight at 4℃ 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 place the precoated

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plate 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 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 1 hour at 4℃ after covering it with plate lid.
7) Wash the precoated plate 9 times in the same manner above 4).
8) "6, Chromogen" should be taken the required quantity into a disposable test tube. Then, pipette 100μL from the test tube into the wells. Please avoid to return the rest of test tube into "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 100μL 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 450nm.
The measurement shall be done within 30minutes after the addition of "7, Stop solution".

SPECIAL ATTENTION

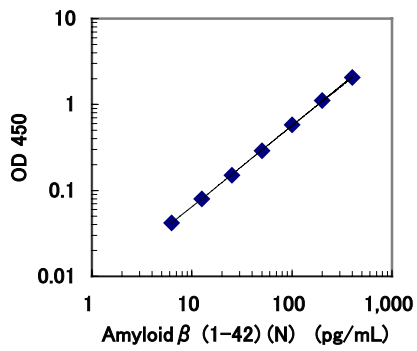
1) Test samples should be measured soon after the collection. In case of the storage of test samples, they should be stored under frozen conditions and do not repeat freeze/thaw cycles. Thaw the test samples at low temperature and mix them completely before measurement.
2) Test samples should be diluted with "4, EIA buffer", if the need arises.
3) The measurement of test samples and standard in duplicate 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)
400	2.127
200	1.182
100	0.645
50	0.356
25	0.217
12.5	0.147
6.25	0.109
0 (Test Sample Blank)	0.067



＊ 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

Specimen	Titer (X)	Measurement Value (pg/mL)	Theoretical Value (pg/mL)	%
10% FCS added RPMI-1640	2	76.24	104.15	73.2
	4	51.38	52.72	97.5
	8	27.03	27.17	99.5
	16	14.10	13.66	103.2
Human Plasma (EDTA)	2	37.95	102.59	37.0
	4	27.81	50.00	55.6
	8	15.50	25.00	62.0
	16	8.10	12.50	64.8
Human Cerebrospinal fluids	4	39.88	50.00	79.8
	8	18.11	25.97	69.7
	16	8.84	12.72	69.5

2. Added Recovery Assay

Specimen	Theoretical Value (pg/mL)	Measurement Value (pg/mL)	%
10% FCS added RPMI-1640 (x4)	101.16	69.53	68.7
	51.16	39.59	77.4
	26.16	19.03	72.8
Human Plasma (EDTA) (x10)	100.00	57.45	57.4
	50.00	27.68	55.4
	25.00	12.41	49.7
Human Cerebrospinal fluids (x10)	50.00	43.11	86.2
	25.00	20.19	80.8
	12.50	9.61	76.9

3. Inter - Assay

Measurement Value (pg/mL)	SD value	CV value (%)	n
210.48	9.13	4.3	23
51.62	2.16	4.2	23
11.74	0.84	7.2	23

4. Intra - Assay

Measurement Value (pg/mL)	SD value	CV value (%)	n
210.77	8.10	3.8	31
52.39	2.58	4.9	31
12.02	1.10	9.2	31

5. Specificity

Compound	Cross Reactivity
Human Aβ (1-42)	100.0%
Human Aβ (1-40)	≦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

2.98 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℃. 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 contact your skin and clothes with "7, Stop solution" and pay attention to the disposal of "7, Stop solution".
4. "1, Precoated plate" and "3, Standard" contain sodium azide. Therefore, dispose these materials after diluting them with large quantity of water to avoid the production of explosive metallic azide.
5. The precipitation may grow 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 different lot or different kit.
8. Do not use the reagents expired.
9. This kit is for research purpose only. Do not use for clinical diagnosis.

STORAGE AND THE TERM OF VALIDITY

Storage Condition : 2 ~ 8℃
The term of validity : 12 months
(The expiry date is specified in outer box.)

REFERENCE

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3. Saido T.C., Iwatsubo T., Mann D.M.A., Shimada H., Ihara Y., and Kawashima S. Dominant and differential deposition of distinct β-amyloid peptide species, Aβ N3(pE), in senile plaques. Neuron 14, 457-466, 1995.
4. Horikoshi Y., Mori T., Maeda M., Kinoshita N., Sato K., Yamaguchi H., Aβ N-terminal-end specific antibody reduced β-amyloid in Alzheimer-model mice. Biochem. Biophys. Res. Commun. 325: 384-387, 2004

Version
040915 Established
051003 Revised (Added Code No. 27729 and INTENDED USE)

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