Code No. 27720

Mouse/Rat Amyloid β (1-40) High Specific Assay Kit - IBL

INTRODUCTION

Alzheimer's disease was first reported by the German neuropathologist A. Alzheimer in 1907, and it is now the most common cause of senile dementia. The numerous senile plaques that occur in the brain in Alzheimer's disease are formed by amyloid beta-protein (Aβ). Aβ is a peptide composed of 40 to 42 (43) amino acids, and is said to be cleaved out of the precursor protein APP (a protein composed of 695, 751, or 770 amino acids) by the action of β- or γ-secretase. (Reference 1)
In addition, the presence of numerous variant Aβ molecules has been demonstrated in the culture fluid of mouse neuroblastoma cells transfected with cDNA coding human amyloid precursor protein (APP). (Reference 2)
Furthermore, in 1995 Saido et al. discovered another type of Aβ peptide, Aβ (N3pE), that is predominant in senile plaques and differed from any type discovered until then. The molecule starts with a molecule in which the 3rd glutamine has been converted to proglutamine by an intramolecular dehydration reaction. (Reference 3)
This product is capable of measuring mouse and rat Aβ1-40, which retains the N-terminal side. It does not detect p3, which is said to be produced by the action of α or γ secretase.
This product is also useful for measuring the following samples accurately.
・Brain extract of mouse or rat that possesses the mouse- or rat-derived APP gene.
・The supernatant of cultured cells that synthesize mouse- or rat-derived Aβ.
・Blood sample (serum or plasma) of mouse or rat.
Since this product hardly cross-reacts with human Aβ, it is useful for the measurement of mouse and rat Aβ1-40, when human Aβ is intermingled with the measurement sample.

IBL Amyloid β Product Lines:

Code No.	Name	Volume
27711	Human Amyloid β (1-42) Assay Kit - IBL	96 Well
27712	Human Amyloid β (1-42) (N) Assay Kit - IBL	96 Well
27713	Human Amyloid β (1-40) Assay Kit - IBL	96 Well
27714	Human Amyloid β (1-40) (N) Assay Kit - IBL	96 Well
27716	Human Amyloid β (N3pE) Assay Kit - IBL	96 Well
27718	Human Amyloid β (1-40) (FL) Assay Kit - IBL	96 Well
27729	Human Amyloid β (1- x) Assay Kit - IBL	96 Well
27720	**Mouse/Rat Amyloid β (1-40) High Specific Assay Kit - IBL**	**96 Well**
27730	Mouse/Rat Amyloid β (1-40) (N) Assay Kit - 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 a coloring agent (Chromogen). The strength of coloring is proportional to the quantities of mouse/rat Aβ (1-40).

MEASUREMENT RANGE

1.56 ～ 100 pg/mL
(0.37 ~23.6 pmol/L, as molecular weight of Aβ (1-40) is 4233.8)

INTENDED USE

■ This kit can be used to measure Mouse/Rat Aβ (1-40) in brain extract, cell culture media supernatant, serum or plasma.
(Actual example 1)
Dilute cell culture media supernatant appropriately with "4, EIA buffer" in the kit, and use as a sample.
(Actual example 2)
Preparation of brain extracted solution
Add 5 volumes of extraction buffer (1% CHAPS in TBS pH7.6) to brain sample, and homogenize them. After thorough homogenization, let stand the emulsion on ice for at least 3 hours. Centrifuge it 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. When measuring this sample, please refer OPERATION MANUAL, 2. Preparation, 3) Preparation of Standard, for detail.).
(Actual example 3)
Dilute serum or plasma appropriately with "4, EIA buffer" in the kit, and use as a sample.
■ When the culture supernatant contains FCS, Aβ (1-40)-like substances are sometimes measured, and it is recommended that a negative control be set.
■ This product is capable of measuring both recombinant and native Mouse/Rat Aβ (1-40).

KIT COMPONENT

1	Precoated plate : Anti- Aβ (35-40) (1A10) Mouse IgG MoAb Affinity Purify	96Well x 1
2	Labeled antibody Conc. : (30X) HRP conjugated Anti-Mouse/Rat Aβ (1-16) Rabbit IgG Fab' Affinity Purify	0.4mL x 1
3	Standard : Mouse/Rat Aβ (1-40)	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℃)
・Paper towel
・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

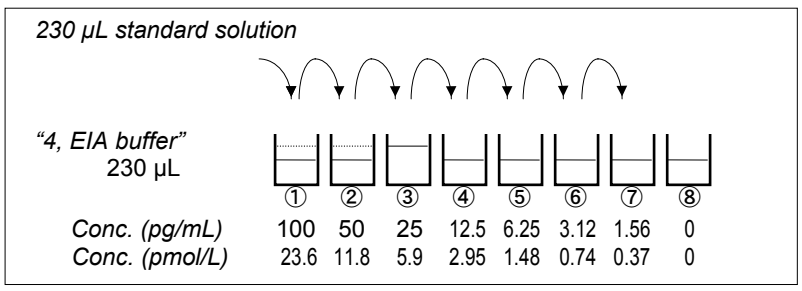
2. Preparation

1) Preparation of wash buffer
"8, Wash buffer Conc." is a concentrated (40X) buffer. 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.)
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 0.5 mL of deionized water into the vial of "3, Standard" and mix it gently and completely. This solution is 200 pg/mL Mouse/Rat Aβ (1-40) standard. However, when measuring brain samples adjusted with extraction buffer, use the same extraction buffer for reconstruction instead of deionized water.
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	100 pg/mL
Tube-2	50 pg/mL
Tube-3	25 pg/mL
Tube-4	12.5 pg/mL
Tube-5	6.25 pg/mL
Tube-6	3.12 pg/mL
Tube-7	1.56 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 100 pg/mL and 1.56 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.

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℃ with plate lid				
Washing 7 times				
Labeled Antibody	100 µL	100 µL	100 µL	-
Incubation for 60 minutes 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 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℃ 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 plate washer, after 4 times washing with plate washer, washing with above washing bottle must be repeated 3 times.
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℃ 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 do not return the rest in 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 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 Blank.
The measurement shall be done within 30 minutes after addition of “7, Stop solution”.

SPECIAL ATTENTION

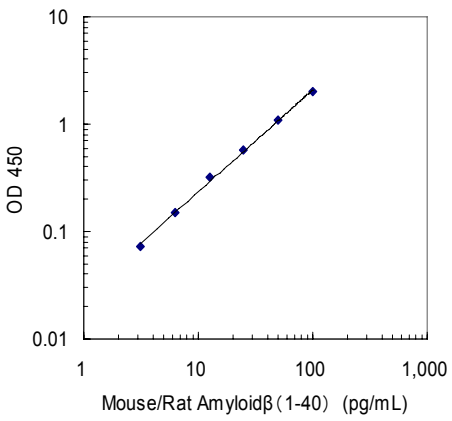
1) Test samples should be measured soon after the collection. For storage of test samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before measurement.
2) Test samples should be diluted with “4, EIA buffer”, if the need 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)
100	2.086
50	1.151
25	0.645
12.5	0.390
6.25	0.224
3.13	0.147
1.56	0.113
0 (Test Sample Blank)	0.074



* 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)	%
Rat Plasma (EDTA) (SD)	4	12.16	13.54	89.8
	8	6.36	7.21	88.2
	16	3.41	3.95	86.3
Rat Serum (SD)	4	9.50	13.92	68.2
	8	4.47	6.38	70.1
	16	2.34	2.87	81.5
Rat brain extract (SD)	16	13.84	14.84	93.3
	32	7.99	8.85	90.3
	64	4.34	4.63	93.7
Mouse Plasma (EDTA) (BALB/c)	4	21.58	25.13	85.9
	8	11.33	14.30	79.2
	16	5.81	7.38	78.7
Mouse Serum (BALB/c)	4	16.75	17.47	95.9
	8	7.20	7.97	90.3
	16	3.35	3.84	87.2
Mouse brain extract (BALB/c)	4	38.73	39.56	97.9
	8	24.12	21.57	111.8
	16	13.03	12.34	105.6

2. Added Recovery Assay

Specimen	Theoretical Value (pg/mL)	Measurement Value (pg/mL)	%
Rat Plasma (EDTA) (SD) (x4)	16.62	11.95	71.9
	10.37	8.21	79.2
	7.24	5.98	82.6
Rat Serum (SD) (x4)	14.44	7.90	54.7
	11.31	7.59	67.1
	9.75	6.79	69.6
Rat brain extract (SD) (x8)	30.85	28.26	91.6
	27.73	22.56	81.4
	26.16	21.30	81.4
Mouse Plasma (EDTA) (BALB/c) (x4)	28.65	31.39	109.6
	25.53	24.76	97.0
	23.97	21.61	90.2
Mouse Serum (BALB/c) (x4)	19.23	15.80	82.2
	16.10	14.40	89.4
	14.54	12.38	85.1
Mouse brain extract (BALB/c) (x8)	22.86	22.37	97.9
	16.61	16.15	97.2
	13.49	12.47	92.4

3. Intra - Assay

Measurement Value (pg/mL)	SD value	CV value (%)	n
68.58	2.48	3.6	24
21.10	0.49	2.3	24
4.07	0.20	4.9	24

4. Inter - Assay

Measurement Value (pg/mL)	SD value	CV value (%)	n
69.50	3.02	4.3	24
21.77	1.27	5.8	24
4.04	0.23	5.7	24

5. Specificity

Compound	Cross Reactivity
Mouse/Rat A β (1-40)	100%
Human A β (1-40)	0.8%

6. Sensitivity

0.28 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 have your skin and clothes contact “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 production of explosive metallic azide.
5. The 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℃
The term of validity : 12 months
(The expiry date is specified in 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.

Version
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