Code No. 27725

Human Amyloidβ Oligomers (82E1-specific) Assay Kit - IBL

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease which Amyloid beta (Aβ) peptide accumulates in a brain and the predominant symptom is a decline of cognitive functions. It has been generally thought that Aβ which condenses and become insoluble fiber (fibril) forms the senile plaque and this denatures neurons and develops the symptoms of the disease. (This is called the 'amyloid hypothesis'.) However, 'oligomer hypothesis' is becoming potent these days that the disease is developed by decline in cognitive function because of the soluble Aβ oligomer which is a middle object of a condensation process has strong synaptic injurious effects. It is reported that when the Aβ oligomer which is secreted from the cell or is extracted from AD patient's brain is injected into rat brain, it causes synaptic dysfunction and study memory loss. Moreover, it is also actually observed that the Aβ oligomers are increasing in brains of AD patients compared with healthy individuals.

PRINCIPLE

This kit is a solid phase sandwich ELISA. The primary reaction of sample and enzyme labeled antibody is performed on the U-plate for primary incubation, this incubated mixture is added to the precoated plate and the secondary reaction is performed. After washing removal of the elements which were not combined with coating antibodies, Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The strength of coloring reflects the amount of molecules which bind to anti-human Aβ (N) (82E1) mouse monoclonal antibodies, recognize the N-terminus of human Aβ specifically, with two or more epitopes. Measurement result is calculated in molarity as a relative value standardized on dimers of Aβ (1-16).

MEASUREMENT RANGE

18.98 – 1,215 pmol/L

INTENDED USE

- This ELISA kit can detect human Aβ molecules which bind to anti-human Aβ (N) (82E1) mouse MoAb with two or more epitopes (oligomers, polymers with a protein etc.) in serum, EDTA-plasma and brain tissue extract.
- Since the result is calculated in molarity standardized on Aβ dimers, it is not in direct proportion to the weight or the number of detected various molecules.

KIT COMPONENT

1	Precoated plate : Anti-Human Aβ (N) (82E1) Mouse IgG MoAb Affinity Purify	96Well x 1
2	Labeled antibody Conc. : (30X) HRP conjugated Anti- Human Aβ (N) (82E1) Mouse IgG MoAb Affinity Purify	0.1mL x 1
3	Standard : Human Aβ (1-16) dimer	0.5mL x 2
4	EIA buffer : 1% BSA, 0.05% Tween20 in PBS	30mL x 1
5	Solution for Labeled antibody : 1% BSA, 0.05% Tween20 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
9	U-Plate for Primary incubation (with a sticker attached)	96Well x 1

OPERATION MANUAL

1. Materials needed but not supplied

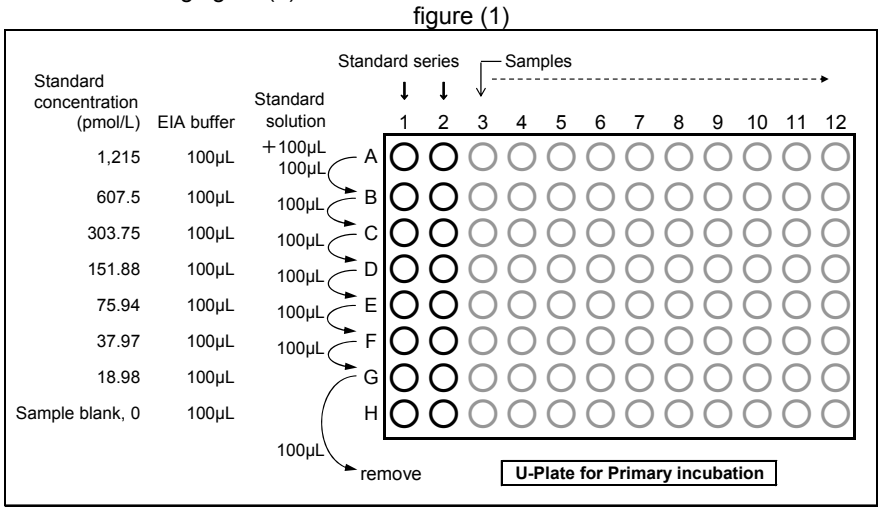
- Plate reader (450nm)
- Micropipette and tip
- Graduated cylinder and beaker
- Deionized water
- Refrigerator (as 4°C)
- Graph paper (log/log)
- Paper towel
- Washing bottle for precoated plate
- Disposable test tube for “2, Labeled antibody Conc.” and “6, Chromogen”

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. (“2, Labeled antibody Conc.” is colored blue.)
Example)
In case one strip (8 wells) is used, the required quantity of Labeled antibody is 160 µL. (Dilute 6 µL of “2, Labeled antibody Conc.” with 174 µL of “5, Solution for Labeled antibody” and mix it. And use the resulting solution by 20 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°C in firmly sealed vial. It is usable until the expiry date.
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 2,430 pmol/L of Human Aβ dimer standard.
4) Dilution of Standard
Dilution of standard is performed on “9, U-Plate for Primary incubation”. As shown in a figure (1), one strip (A - H) of “9, U-Plate for Primary incubation” is used for a dilution series of standard. Since we recommend duplication measurement to construct the standard curve, please prepare two sequences of standard dilution series similarly. For example, A1 - H1 and A2 - H2.
a) Put 100 µL of “4, EIA buffer” into each well from A to H.
b) Add 100 µL of the 2,430 pmol/L standard solution to well-A, and mix it gently (about 10 times pipetting). Then, put 100 µL of the mixed solution in well-A into well-B.
c) In this way, dilute standard solution until well-G sequentially. Then, remove 100 µL of diluted standard solution from well-G.
d) These7 points from well-A to well-G are the standard solution series from 1,215 pmol/L to 18.98 pmol/L, and well-H is a test sample blank as 0 pmol/L.

See following figure (1).

figure (1)



5) Dilution of test sample
Test samples should be diluted with “4, EIA buffer” as necessary.
If the measurement value of samples may not be estimated in advance, the pre-assay with several different dilutions will be recommended to determine the proper dilution of samples.

6) Preparation of Precoated plate
Remove strips which won’t be used for the test from a holder. (They will be applicable until the expiry date when they are sealed in the bag which into they went and they are refrigerated.)
Determine and specify each well for diluted standards, test samples, test sample blank and reagent blank.

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.

		Test Sample	Standard	Test Sample Blank	Reagent Blank
U-Plate for Primary incubation	Sample	Test sample 100 µL	Diluted standard (Well-A – Well-G) 100 µL	EIA buffer (Well-H) 100 µL	EIA buffer 120 µL
	Labeled Antibody	20 µL	20 µL	20 µL	—
	Incubation for 60 minutes at 4°C with plate lid				
Precoated plate	Solution after the primary incubation	100 µL	100 µL	100 µL	100 µL
	Incubation for 60 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) Set the wells for reagent blank on “9, U-Plate for Primary incubation”. Put 120 µL of “4, EIA buffer” into the reagent blank wells.
2) Set the wells for test samples on “9, U-Plate for Primary incubation”. Then, put 100 µL of test samples into each appropriate well.
3) Add 20 µL of labeled antibody solution to each well of diluted standards, test samples and test sample blank. (The solution in wells which added labeled antibody is turned blue.) While adding labeled antibody, mix the solution by pipetting in each well.
4) Incubate the “9, U-Plate for Primary incubation” for 60 minutes at 4°C after covering it with plate lid.
5) Take each 100 µL of solution after primary incubation from wells of test samples, diluted standards, test sample blank and reagent blank on “9, U-Plate for Primary incubation”, then put them into the appropriate wells of “1, Precoated plate”. *Before taking solution from wells, mix and homogenize them by pipetting in each well.*
Seal the used wells of “9, U-Plate for Primary incubation” with an attached sticker etc. not to contaminate unused wells for storage.
6) Incubate the “1, Precoated plate” for 60 minutes at 4°C after covering it with plate lid.
7) Wash each well of the precoated plate vigorously with wash buffer using the washing bottle. Then, fill each well with wash buffer and leave the precoated plate laid 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.
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”.
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

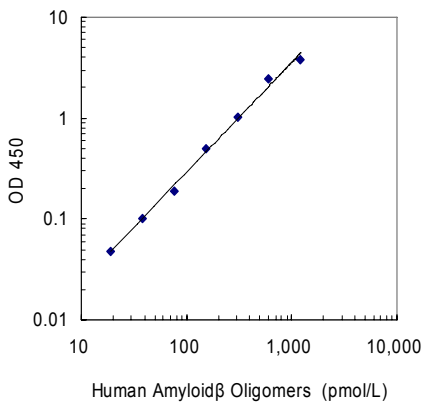
1) Test samples should be measured soon after collection. For the storage of test samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before measurement.
2) Test samples should be diluted with “4, EIA buffer”, if the need 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. (pmol/L)	Absorbance (450nm)
1,215	3.778
607.5	2.456
303.75	1.036
151.88	0.494
75.94	0.195
37.97	0.105
18.98	0.052
0 (Test Sample Blank)	0.004



＊ 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 (pmol/L)	Theoretical Value (pmol/L)	%
RPMI-1640 FCS (-)	2	504.12	607.50	83.0
	4	266.47	303.75	87.7
	8	133.73	151.88	88.0
Human Serum	2	612.35	691.84	88.5
	4	309.10	353.20	87.5
	8	153.07	177.73	86.1
Human Plasma (EDTA)	2	632.27	670.54	94.3
	4	294.36	341.62	86.2
	8	150.86	173.73	86.8
Human Cerebrospinal fluid	4	262.76	303.75	86.5
	8	126.17	151.88	83.1
	16	67.54	75.94	88.9
Brain tissue Extract (Tg mouse)	2	521.97	643.18	81.2
	4	280.43	345.44	81.2
	8	149.04	192.35	77.5

*CHAPS buffer was used for brain tissue extraction

2. Added Recovery Assay

Specimen	Theoretical Value (pmol/L)	Measurement Value (pmol/L)	%
RPMI-1640 FCS (-) (x2)	151.88	128.01	84.3
	75.94	64.23	84.6
	37.97	36.97	97.4
Human Serum (x2)	117.42	104.26	88.8
	98.43	88.96	90.4
	88.94	83.49	93.9
Human Plasma (EDTA) (x2)	100.18	82.75	82.6
	81.19	73.84	90.9
	71.70	65.98	92.0
Human Cerebrospinal Fluid (x4)	151.88	131.69	86.7
	75.94	69.24	91.2
	37.97	36.97	97.4
Brain tissue Extract (Tg mouse) (x4)	117.44	99.64	84.8
	79.44	64.93	81.7
	60.44	56.73	93.9

3. Intra - Assay

Measurement Value (pmol/L)	SD value	CV value (%)	n
314.24	15.62	5.0	24
88.32	2.56	2.9	24
33.66	1.83	5.4	24

4. Inter - Assay

Measurement Value (pmol/L)	SD value	CV value (%)	n
310.02	9.75	3.1	5
76.92	3.99	5.2	5
27.39	2.75	10.0	5

5. Specificity

Compound	Cross Reactivity
Aβ (1-16) dimer peptide	100 %
Aβ (1-40) peptide	0.27 %
Aβ (N3pE-40) peptide	≦ 0.1%
Aβ (N3pE-42) peptide	≦ 0.1%

6. Sensitivity

4.41 pmol/L
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. Xia W, Yang T, Shankar G, Smith IM, Shen Y, Walsh DM, Selkoe DJ. A specific enzyme-linked immunosorbent assay for measuring beta-amyloid protein oligomers in human plasma and brain tissue of patients with Alzheimer disease. Arch Neurol. 2009 Feb;66(2):190-9.
2. Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, Rowan MJ, Selkoe DJ. Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. Nature. 2002, 4;416(6880):535-9.
3. Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, Brett FM, Farrell MA, Rowan MJ, Lemere CA, Regan CM, Walsh DM, Sabatini BL, Selkoe DJ. Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nat Med. 2008, ;14(8):837-42.

Version 1.2.

Made in Japan.