

Code No. 27716

Human Amyloid β (N3pE-42) Assay Kit - IBL

Please read carefully this instruction prior you use this assay kit.*

INSTRUCTIONS FOR USE

This product is for research use only and is not intended for diagnostic use.*

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 A β (N3pE-42).

MEASUREMENT RANGE

7.75 - 496 pg/mL

(1.8 - 115 pmol/L, as molecular weight of A β (N3pE-42) is 4,310)**INTENDED USE**

- Both recombinant and native forms of human A β (N3pE-42) can be detected with the kit.
- Human A β (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 A β (N3pE-42) in these samples.

KIT COMPONENT

1	Precoated plate : Anti- Human A β (38-42) Rabbit IgG Affinity Purify	96Well x 1
2	Labeled antibody Conc. : (30X) HRP conjugated Anti- Human A β N3pE (8E1) Mouse IgG Fab'Affinity Purify	0.4mL x 1
3	Standard : Human A β (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)
- Graduated cylinder and beaker
- Refrigerator (as 4°C)
- Paper towel
- Plate washer or washing bottle*
- 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

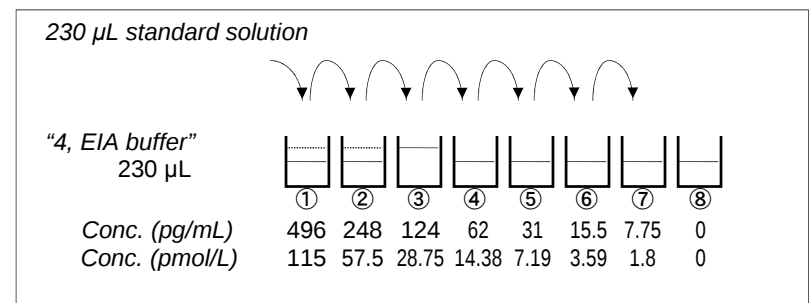
2. Preparation

- 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 μ L. (Dilute 30 μ L of "2, Labeled antibody Conc." with 870 μ L of "5, Solution for Labeled antibody" and mix it. And use the resulting solution by 100 μ L 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 deionized water into the vial of "3, Standard" and mix it gently and completely. This solution is 992 pg/mL Human A β (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 μ L 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 μ L of Standard solution into tube-1 and mix it gently. Then, put 230 μ L 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 A β (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 μ L	Diluted standard (Tube 1~7) 100 μ L	EIA buffer (Tube-8) 100 μ L	EIA buffer 100 μ L
Incubation overnight at 4°C with plate lid				
4 times (wash buffer more than 350 μ L) (Refer to No. 8 and 9 described in OPERATING PRECATION.)*				
Labeled Antibody	100 μ L	100 μ L	100 μ L	-
Incubation for 60 minutes at 4°C with plate lid				
5 times (wash buffer more than 350 μ L) (Refer to No. 8 and 9 described in OPERATING PRECATION.)*				
Chromogen	100 μ L	100 μ L	100 μ L	100 μ L
Incubation for 30 minutes at room temperature (shielded)				
Stop solution	100 μ L	100 μ L	100 μ L	100 μ L
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 μ L each of "4, EIA buffer" into the wells.
- 2) Determine wells for test sample blank, test sample and diluted standard. Then, put 100 μ L 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 (Refer to No. 8 and 9 described in OPERATING PRECATION.)*
Wash the plate with the prepared wash buffer and remove all liquid.
- 5) Pipette 100 μ L 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 (Refer to No. 8 and 9 described in OPERATING PRECATION.)*
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 μ L 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 μ L 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 storage of 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" contained in this kit.
- 3) Duplicate measurement of test samples and standards is recommended.
- 4) Standard curve should run for each assay.
- 5) Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
- 6) All reagents should be brought to room temperature (R.T.) and mixed completely and gently before use. After mixing them, make sure of no change in quality of the reagents.
- 7) Use only "8, Wash buffer conc." contained in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.
- 8) Using a plate washer is recommended (wait time zero second). It should be washed by a plate washer immediately after each reaction. If you use a washing bottle instead of a plate washer, after filling wash buffer in each well,

immediately turn the plate upside down and shake it off to completely remove the wash buffer. Repeat the number of times of wash defined in a table for measurement procedure described in section 3. It should be properly washed off as instructed in order to avoid any insufficient wash.

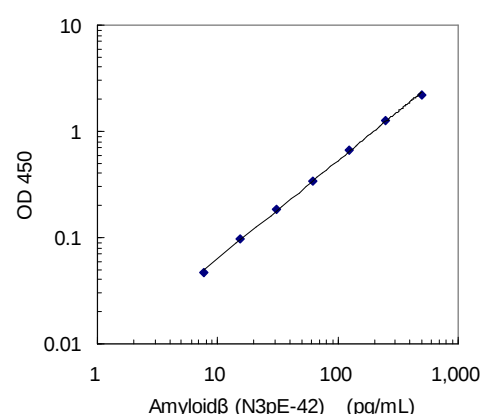
- 9) Carefully tap the plate against a clean paper towel without contacting with inside of each well to completely remove the washing buffer after repeated the determined number of wash.
- 10) "6, Chromogen - TMB solution" should be stored in the dark due to its sensitivity against light. It should be also avoided contact with metals. Required quantity should be prepared into a collecting container for each use.
- 11) After adding TMB solution into the wells, the liquid in the wells gradually changes the color in blue. In this process the plate should be in dark. Remained TMB solution in the collecting container should not be returned into the original bottle of TMB solution to avoid contamination.
- 12) Measurement of O.D. 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 A β (N3pE-42)	100.0%
Human A β (1-40)	$\leq 0.1\%$
Human A β (1-42)	$\leq 0.1\%$
Human A β (1-43)	$\leq 0.1\%$
Human A β (17-40), (P3 Form)	$\leq 0.1\%$
Rat/Mouse A β (1-40)	$\leq 0.1\%$
Rat/Mouse A β (1-42)	$\leq 0.1\%$

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 4.

June 2017 *