Code No. 27731

Human sAPP, Total (highly sensitive) Assay Kit - IBL

INTRODUCTION

Alzheimer's disease (AD) was first reported by A. Alzheimer, a German neuropathologist in 1907 and is considered as a major factor of dementia. It is known that Amyloid β (Aβ; which is major constituent of senile plaque) is cleaved from Amyloid Precursor Protein (APP; which exists in three main isoforms, APP695, APP751, and APP770) by β-secretase and subsequent γ-secretase (ref. 1). The production of soluble APPβ (sAPPβ) by β-secretase cleavage corresponds to Aβ production accordingly, so it is desired to measure sAPPβ in parallel with Aβ. In addition, it is reported that APP gene mutation exists in early-onset familial Alzheimer's disease patient. Swedish mutation, one of the APP gene mutations, is a double mutation at positions -1 to -2 from the β-secretase cleavage site (Lys670→Asn and Met671→Leu). And further, it is reported that Swedish mutation elevates Aβ40 and Aβ42 production (ref. 2), and that the mutation is utilized in establishment of transgenic mice (ref. 3). The measuring sAPPβ in Swedish type is useful for research of AD as well as in wild type. On the one hand, it is considered that in the metabolic pathway of APP, APP is first cleaved by α-secretase rather than β-secretase normally to produce soluble APPα (sAPPα) and subsequently P3 is cleaved from the remaining C-terminal fragment by γ-secretase. In recent research, there are several attempts to apply the inhibitor of β-secretase and the activation of α-secretase for AD treatment.
This kit can measure human soluble APP in samples as a total amount including sAPPα and sAPPβ.

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 total quantities of human total sAPP.

MEASUREMENT RANGE

0.39 - 25 ng/mL

INTENDED USE

- This IBL's assay kit is capable for the quantitative determination human total sAPP in serum, EDTA plasma, cerebrospinal fluid and cell culture supernatant as a total amount.
- The recommended dilution for serum samples is more than 4-fold, for EDTA-plasma samples is more than 2-fold and for cerebrospinal fluids is more than 16-fold.

KIT COMPONENT

1	Precoated plate	: Anti-Human APP (R12A1) Mouse IgG MoAb Affinity Purify	96Well x 1
2	Labeled antibody Conc.	: (30X) HRP conjugated Anti- Human APP (R101A4) Mouse IgG MoAb Fab' Affinity Purify	0.4mL x 1
3	Standard	: Recombinant Human sAPPα Protein	0.5mL x 2
4	EIA buffer	:	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

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"

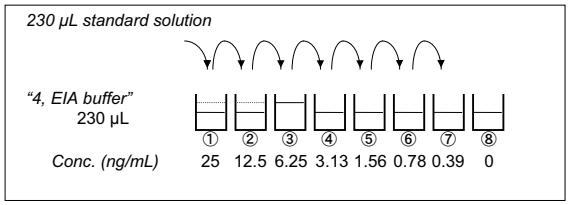
2. Preparation

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 50 ng/mL Human sAPP 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	25 ng/mL	
Tube-2	12.5 ng/mL	
Tube-3	6.25 ng/mL	
Tube-4	3.13 ng/mL	
Tube-5	1.56 ng/mL	
Tube-6	0.78 ng/mL	
Tube-7	0.39 ng/mL	
Tube-8	0 ng/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 25 ng/mL and 0.39 ng/mL. Tube-8 is the test sample blank as 0 ng/mL.

See following picture.



5) Dilution of test sample
Test samples need to be diluted with "4, EIA buffer" attached with this kit accordingly .
If the concentration of human sAPP 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				
Washing 7 times				
Labeled Antibody	100 µL	100 µL	100 µL	-
Incubation for 30 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) 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) 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. <u>This procedure must be repeated more than 7 times.</u> 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.
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 30 minutes at 4°C after covering it with plate lid.
7) Wash the precoated plate 9 times in the same manner as 4).
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

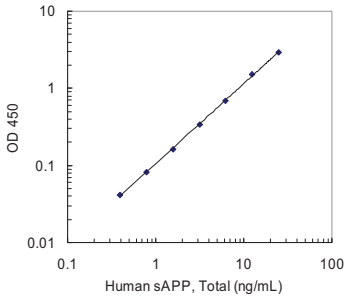
the test samples at a low temperature and mix them completely before measurement.
2) Test samples should be diluted with “4, EIA buffer”, if the need arises.
3) Duplicate measurement of test samples and standard is recommended.
4) Use test samples in neutral pH range. The contaminations of organic solvent 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. (ng/mL)	Absorbance (450nm)
25	2.958
12.5	1.530
6.25	0.693
3.13	0.343
1.56	0.162
0.78	0.083
0.39	0.043
0 (Test Sample Blank)	0.001



＊ 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 (ng/mL)	Theoretical Value (ng/mL)	%
10 % FCS added RPMI-1640	2	10.90	12.50	87.2
	4	5.65	6.25	90.4
	8	2.63	3.13	84.0
Human Serum	4	21.26	22.89	92.9
	8	11.64	11.25	103.5
	16	5.81	5.47	106.2
Human Plasma (EDTA)	4	14.87	17.19	86.5
	8	7.74	8.43	91.8
	16	3.66	4.23	86.5
Human Cerebrospinal fluids	4	24.00	25.87	92.8
	8	15.42	14.20	108.6
	16	9.05	9.12	99.2

2. Added Recovery Assay

Specimen	Theoretical Value (ng/mL)	Measurement Value (ng/mL)	%
10 % FCS added RPMI-1640 (x2)	12.50	12.52	100.2
	6.25	7.37	117.9
	3.13	3.51	112.1
Human Serum (x8)	23.22	23.45	101.0
	16.97	17.13	100.9
	13.85	13.21	95.4
Human Plasma (EDTA) (x8)	13.31	13.35	100.3
	10.19	9.98	97.9
	8.63	8.14	94.3
Human Cerebrospinal fluids (x8)	26.34	26.94	102.3
	23.21	22.24	95.8
	21.65	20.11	92.9

3. Intra – Assay

Measurement Value (ng/mL)	SD value	CV value (%)	n
11.71	0.53	4.5	24
3.92	0.17	4.3	24
1.36	0.06	4.4	24

4. Inter - Assay

Measurement Value (ng/mL)	SD value	CV value (%)	n
11.83	0.46	3.9	4
3.85	0.50	13.0	4
1.30	0.10	7.7	4

5. Specificity

Compound	Cross Reactivity
Human sAPPα	100 %
Human sAPPβ-wild type	100 %
Human sAPPβ-swedish type	100 %

6. Sensitivity

0.06 ng/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.
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3. Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang F, Cole G. Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. Science. 1996 Oct 4;274(5284):99-102.

Version 1.2

Made in Japan.

＜Distributed by＞

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