

Code No. 27740

Human α -Synuclein Assay Kit - IBL**INTRODUCTION**

α -Synuclein is a 14.5kDa, soluble form protein which consists of 140 amino-acid residue and it is known as an intrinsically disordered protein. It is mainly expressed in cerebral neocortex, hippocampus, nigra, thalamus and metencephalon. Majority of it exist in presynaptic terminal and nucleus of neuron cells and it is considered to involve with signal regulatory system of synaptic vesicle.

α -Synuclein is main structural component of Lewy body which is an inclusion body exists inside of neuron cells in neurodegenerative diseases called "Synucleinopathy" such as Parkinson's disease, dementia with Lewy body and multiple system atrophy.

It is considered that excessive accumulation of α -Synuclein is main cause of synucleinopathy due to inducing of shedding of dopamine-producing cells. The concentration of α -Synuclein in human blood or human CSF can be quantitatively measured by this ELISA kit.

PRINCIPLE

This kit is a solid phase sandwich ELISA using 2 kinds of highly specific antibodies. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The strength of coloring is proportional to the quantities of Human α -Synuclein.

MEASUREMENT RANGE

0.16 - 10.00ng/mL

INTENDED USE**For research use only, not for use in diagnostic procedures.**

This IBL's assay kit is capable for the quantitative determination Human α -Synuclein in serum, EDTA plasma, CSF and cell culture supernatant.

Recommended dilution ratio for;

- (1) Normal human serum and EDTA plasma samples is around 2 to 4-fold.
- (2) Normal human CSF samples is around 2 to 8-fold.

KIT COMPONENT

1	Precoated plate	: Anti-Human α -SNCA (3G11) Mouse IgG MoAb	96Well x 1
2	Labeled antibody Conc.	:	
		(30X) HRP conjugated Anti-Human (5G12) Mouse IgG Fab'	0.4mL x 1
3	Standard	: Recombinant Human α -SNCA	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
- 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 1950 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-fold 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 applying labeled antibody.

The remaining "2, Labeled antibody Conc." should be stored at 2 - 8°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 20.00ng/mL Human α -Synuclein standard.

4) Dilution of Standard

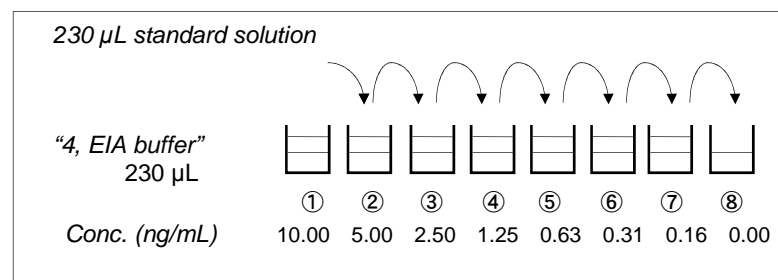
Prepare 8 tubes for dilution of "3, Standard". Put 230 μ L each of "4, EIA buffer" into the tube.

Specify the following concentration of each tube.

Tube-1	10.00 ng/mL
Tube-2	5.00 ng/mL
Tube-3	2.50 ng/mL
Tube-4	1.25 ng/mL
Tube-5	0.63 ng/mL
Tube-6	0.31 ng/mL
Tube-7	0.16 ng/mL
Tube-8	0.00 ng/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 10.00 ng/mL and 0.16 ng/mL. Tube-8 is the test sample blank as 0.00 ng/mL.

See following picture.



5) Dilution of test sample

Test samples should be diluted with "4, EIA buffer" suitably.

Recommended dilution ratio for;

- (1) Normal human serum and EDTA plasma samples is around 2 to 4-fold.
- (2) Normal human CSF samples is around 2 to 8-fold.

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 O/N at 2 - 8°C with plate lid				
4 times (wash buffer more than 350 μ L) *				
Labeled Antibody	100 μ L	100 μ L	100 μ L	-
Incubation for 60 minutes at 2 - 8°C with plate lid				
5 times (wash buffer more than 350 μ L) *				
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 2 - 8°C after covering it with plate lid.
- 4) 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 2 - 8°C after covering it with plate lid.
- 7) Wash the plate with the prepared wash buffer and remove all liquid. *
- 8) Take the required quantity of "6, Chromogen" and put it into a disposable test tube. Then, pipette 100 μ L from the test tube into every well. Please do not return the rest of used Chromogen in the test tube into "6, Chromogen" bottle in order to avoid contamination.
- 9) Incubate the precoated plate for 30 minutes at room temperature in the dark. The solution of Chromogen will turn blue.
- 10) Add 100 μ L of "7, Stop solution" to all wells. Mix the solution by tapping the side of precoated plate. The solution 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 solution. 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

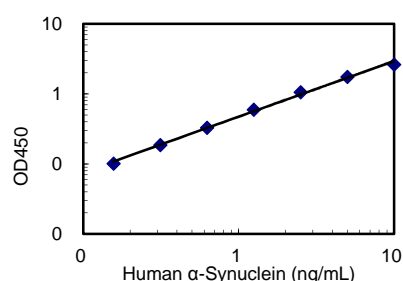
- 1) Test samples should be measured soon after collection. For the storage of test samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before measurement.
- 2) Test samples should be diluted with "4, EIA buffer", suitably.
- 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 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. Avoid contact of Chromogen 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

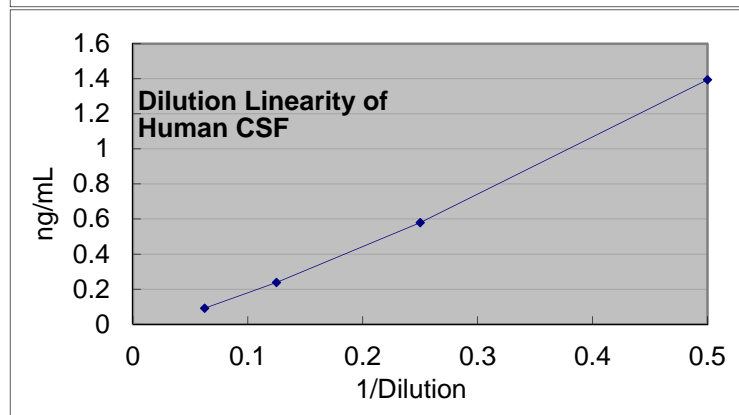
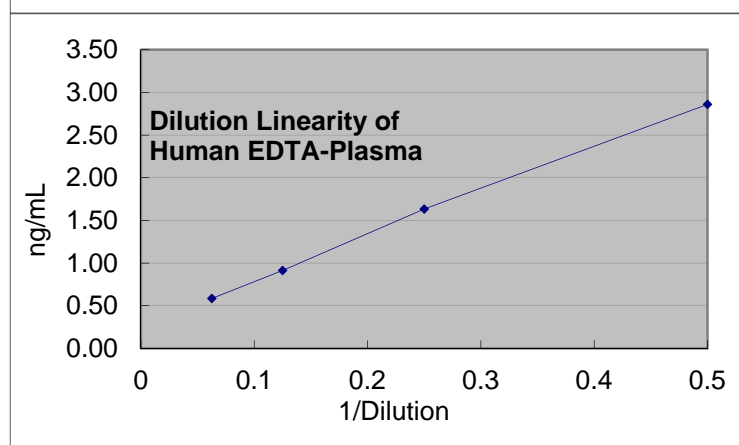
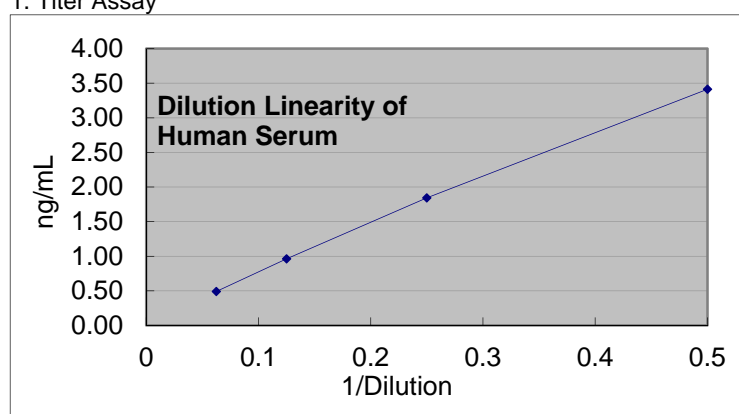
Conc. (ng/mL)	Absorbance (450nm)
10.00	2.687
5.00	1.819
2.50	1.122
1.25	0.656
0.63	0.390
0.31	0.247
0.16	0.162
0.00 (Test Sample Blank)	0.060



* The typical standard curve is shown above. This curve cannot be used to derive test results. Please run a standard curve for each assay.

PERFORMANCE CHARACTERISTICS

1. Titer Assay



2. Added Recovery Assay

Specimen	Theoretical Value (ng/mL)	Measured Value (ng/mL)	%
Human Serum x4	5.95	3.94	66.2
	3.45	2.39	69.2
	2.20	1.71	77.9
Human Plasma (EDTA) x4	3.03	2.01	66.4
	1.78	1.19	66.6
	1.16	0.85	73.7
Human CSF x4	5.71	4.67	81.9
	3.21	2.45	76.3
	1.96	1.65	84.2
Medium (with 10% FBS) x2	5.00	4.38	87.5
	2.50	2.30	92.1
	1.25	1.02	81.4

3. Intra - Assay

Mean Value (ng/mL)	SD (ng/mL)	CV (%)	n
5.38	0.39	7.2	24
2.77	0.10	3.7	24
1.30	0.08	6.0	24

4. Inter - Assay

Mean Value (ng/mL)	SD (ng/mL)	CV (%)	n
4.48	0.35	7.8	5
2.25	0.20	8.9	5
1.04	0.09	9.1	5

5. Specificity

Substance	Cross-Reactivity
Human α -Synuclein	100%
Human β -Synuclein	N.D.
Human γ -Synuclein	N.D.

6. Sensitivity

0.03ng/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

- All reagents should be stored at 2 - 8°C. All reagents shall be brought to room temperature approximately 30 minutes before use.
- "3, Standard" is lyophilized products. Be careful to open this vial.
- "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".
- Dispose used materials after rinsing them with large quantity of water.
- Precipitation may occur in "2, Labeled antibody Conc.", "4, EIA buffer" or "8, Wash buffer Conc.", however, there is no problem in the performance.
- Wash hands after handling reagents.
- Do not mix the reagents with the reagents from a different lot or kit.
- Do not use expired reagents.
- 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

- Ulmer TS, Bax A, Cole NB, Nussbaum RL (Mar 2005). "Structure and dynamics of micelle-bound human alpha-synuclein". J Biol Chem 280 (10): 9595-9603.
- Alim MA, Hossain MS, Arima K, Takeda K, Izumiyama Y, Nakamura M, Kaji H, Shinoda T, Hisanaga S, Ueda K. (Jan 2002). "Tubulin seeds alpha-synuclein fibril formation.". J. Biol. Chem. 277 (3): 2112-2117.
- Iwai A, Masliah E, Yoshimoto M, Ge N, Flanagan L, de Silva HA, Kittel A, Saitoh T (February 1995). "The precursor protein of non-A beta component of Alzheimer's disease amyloid is a presynaptic protein of the central nervous system". Neuron 14 (2): 467-475.
- Bartels T, Choi JG, Selkoe DJ (August 2011). "alpha-Synuclein occurs physiologically as a helically folded tetramer that resists aggregation". Nature 477 (7362).
- Okochi M, Walter J, Koyama A, Nakajo S, Baba M, Iwatsubo T, Meijer L, Kahle PJ, Haass C (Jan 2000). "Constitutive phosphorylation of the Parkinson's disease associated alpha-synuclein" (free full text). J Biol Chem 275 (1): 390-397.
- Tokuda T, Salem SA, Allsop D, Mizuno T, Nakagawa M, Qureshi MM, Locascio JJ, Schlossmacher MG, El-Agnaf OM. "Decreased alpha-synuclein in cerebrospinal fluid of aged individuals and subjects with Parkinson's disease." Biochem Biophys Res Commun. 2006 Oct 13;349(1):162-6. Epub 2006 Aug 14.

Version 2.

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Made in Japan.