Human a-Synuclein Sandwich ELISA Kit Datasheet

For the quantitative detection of human a-Synuclein in serum and plasma.

General Information

Catalogue Number	KE00191
Product Name	Human a-Synuclein Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	0.312-20 ng/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	6622
SwissProt	P37840

Kit Components & Storage

Microplate - antibody coated 96 - well microplate (8 well × 12 strips)	1 plate	**Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.**
Protein standard - 40 ng/bottle; lyophilized*	2 bottles	
Detection antibody, HRP-conjugated (100X) - 120 μL/vial	1 vial	
Sample Diluent PT 3-dg - 30 mL/bottle	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	3 pieces	

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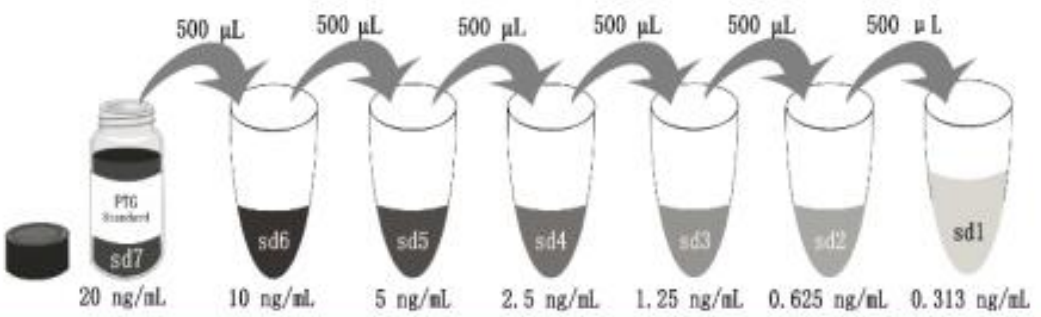
NB: Do not use the kit after the expiration date.
Sample Diluent PT 3-dg is for protein standard and plasma samples.
Detection Diluent is for Detection antibody.
*Add **2 mL** Sample Diluent PT 3-dg in proetin standard. This reconstitution gives a stock solution of 20 ng/mL.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 3-dg	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00191 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The a-Synclein ELISA kit is to be used to detect and quantify protein levels of endogenous a-Synclein. The assay recognizes human a-Synclein. An antibody specific for a-Synclein has been pre-coated onto the microwells. The a-Synclein protein in samples is captured by the coated antibody after incubation. Following extensive washing, another horseradish peroxidase (HRP)-conjugated antibody specific for a-Synclein is added to detect the captured a-Synclein protein. For signal development, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm .

Background

alpha-synuclein (α -syn) is a 14-19 kDa phosphoprotein that is primarily localize to the presynaptic terminals of mature neurons, where it is involved in synaptic function and plasticity. a-syn has drawn intense interest ever since the late 1990s, when the first α -synuclein missense mutation was identified as a cause of familial Parkinson's disease (PD). Aggregated and hyper-phosphorylated forms of α -syn protein are the pathological hallmark of Lewy body disease, which includes Parkinson's disease (PD), diffuse Lewy body disease (DLBD), and Lewy body variant of Alzheimer's disease (LBV).

Sample Preparation

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The plasma samples may require proper dilution to fall within the range of the assay. 1:4 dilution is recommended for the individual samples.

Safety Notes

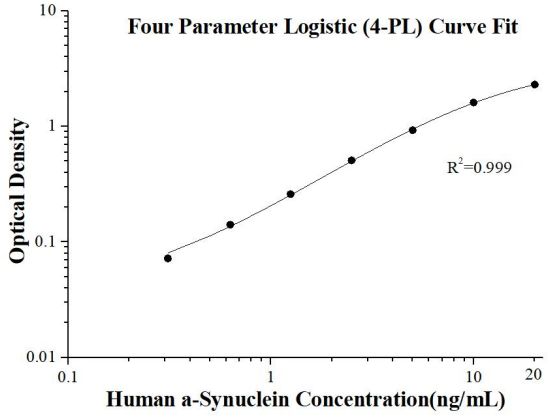
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Detection antibody, HRP-conjugated Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
4	Stop Solution	100 µL	0 min	Do not wash	-
5	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

Example data

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D	Average	Corrected
0	0.093 0.093	0.093	-
0.312	0.164 0.165	0.165	0.072
0.625	0.232 0.236	0.234	0.141
1.25	0.354 0.349	0.352	0.259
2.5	0.592 0.612	0.602	0.509
5	1.009 1.033	1.021	0.928
10	1.725 1.681	1.703	1.61
20	2.355 2.438	2.397	2.304

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Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (ng/mL)	SD	CV%
1	20	9.43	0.41	4.3
2	20	2.27	0.08	3.5
3	20	0.55	0.02	3.6

Inter-assay Precision				
Sample	n	Mean (ng/mL)	SD	CV%
1	24	9.61	0.44	4.6
2	24	2.36	0.10	4.2
3	24	0.57	0.05	8.8

Recovery

The recovery of a-Synuclein spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Human plasma	1:2	109	89-125
	1:4	101	94-109

Sample Values

Plasma samples from healthy volunteers (human) were evaluated for a-Synuclein in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (ng/mL)	Range (ng/mL)
Human plasma (n=6)	13.1	1.8-24.9

Sensitivity

The minimum detectable dose of human a-Synuclein is 45 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

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Linearity

To assess the linearity of the assay, human plasma samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

		Human plasma
1:2	Average% of Expected	100
	Range (%)	-
1:4	Average% of Expected	89
	Range (%)	85-93
1:8	Average% of Expected	90
	Range (%)	81-105
1:16	Average% of Expected	92
	Range (%)	85-100

References

1. Benskey MJ, et al. The contribution of alpha synuclein to neuronal survival and function - Implications for Parkinson's disease. J Neurochem. 137(3):331-59. (2016)
2. Bougea A., et al. Plasma alpha-synuclein levels in patients with Parkinson's disease: a systematic review and meta-analysis. Neurol Sci. 40(5):929-938. (2019)

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