

Human Alpha-synuclein ELISA Kit ab260052

KO VALIDATED Recombinant SimpleStep ELISA

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Overview

Product name Human Alpha-synuclein ELISA Kit

Detection method Colorimetric

Precision Intra-assay

Sample	n	Mean	SD	CV%
Serum	8			2.5%

Inter-assay

Sample	n	Mean	SD	CV%
Serum	3			8.9%

Sample type Serum, Cell Lysate, Cell culture media, Hep Plasma, EDTA Plasma, Cit plasma, Tissue Lysate

Assay type Sandwich (quantitative)

Sensitivity 504 pg/ml

Range 615 pg/ml - 7000 pg/ml

Recovery Sample specific recovery

Sample type	Average %	Range
Serum	117	116% - 118%
Cell culture media	103	90% - 114%
Hep Plasma	106	93% - 115%
EDTA Plasma	118	105% - 129%
Cit plasma	113	113% - 113%

Assay time 1h 30m

**Assay duration**

One step assay

**Species reactivity**

**Reacts with:** Human

**Product overview**

Human Alpha-synuclein ELISA Kit (ab260052) is a single-wash 90 min sandwich ELISA designed for the quantitative measurement of Alpha-synuclein protein in cell culture media, cell lysate, cit plasma, edta plasma, hep plasma, and serum. It uses our proprietary SimpleStep ELISA® technology. Quantitate Human Alpha-synuclein with 504 pg/ml sensitivity.

SimpleStep ELISA® technology employs capture antibodies conjugated to an affinity tag that is recognized by the monoclonal antibody used to coat our SimpleStep ELISA® plates. This approach to sandwich ELISA allows the formation of the antibody-analyte sandwich complex in a single step, significantly reducing assay time. See the SimpleStep ELISA® protocol summary in the image section for further details. Our SimpleStep ELISA® technology provides several benefits:

- Single-wash protocol reduces assay time to 90 minutes or less
- High sensitivity, specificity and reproducibility from superior antibodies
- Fully validated in biological samples
- 96-wells plate breakable into 12 x 8 wells strips

A 384-well SimpleStep ELISA® microplate (**ab203359**) is available to use as an alternative to the 96-well microplate provided with SimpleStep ELISA® kits.

**ASSAY SPECIFICITY**

This kit recognizes both native and recombinant human Alpha-synuclein protein in serum, plasma, and cell culture supernatant, cell and tissue extract samples only.

Urine, saliva, and cerebrospinal fluid samples have not been tested with this kit.

**CROSS REACTIVITY**

Recombinant Tau 441, UCHL-1, beta-tubulin, and Gamma-synuclein were prepared at 7,000 pg/mL and assayed for cross reactivity. No cross-reactivity was observed.

**INTERFERENCE**

Recombinant Tau 441, UCHL-1, and beta tubulin were prepared at 4 ng/mL and tested for interference. No interference was observed.

**SPECIES REACTIVITY**

This kit recognizes human Alpha-synuclein protein.

Other species reactivity was determined by measuring 50% serum samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human serum assayed at the same dilution.

This kit was found to react with rhesus macaque serum.

Reactivity < 3% was determined for the following species:

Mouse

Rat

Cow

Other species reactivity not determined.

**Notes** Alpha-synuclein is a 140- amino acid protein encoded by the SNCA gene, primarily found in neural tissue. It is a member of the synuclein family of proteins, including  $\beta$ -Synuclein and  $\gamma$ -Synuclein. Alpha-synuclein has been found concentrated in the presynaptic nerve terminals of neurons and has been found in the nucleus of neurons. Alpha-synuclein is a major component of Lewy Bodies. This protein appears to play a role in dopamine metabolism and vesicle trafficking.

Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances.  
It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.

**Platform** Pre-coated microplate (12 x 8 well strips)

## Properties

**Storage instructions** Store at +4°C. Please refer to protocols.

Components	1 x 96 tests	1 x 96 tests
10X Human Alpha-synuclein Capture Antibody	1 x 600 $\mu$ l	1 x 600 $\mu$ l
10X Human Alpha-synuclein Detector Antibody	1 x 600 $\mu$ l	1 x 600 $\mu$ l
10X Wash Buffer PT (ab206977)	1 x 20ml	1 x 20ml
5X Cell Extraction Buffer PTR (ab193970)	1 x 10ml	1 x 10ml
Antibody Diluent CPI2	1 x 6ml	1 x 6ml
Human Alpha-synuclein Lyophilized Recombinant Protein (ab51189)	2 vials	2 vials
Plate Seals	1 unit	1 unit
Sample Diluent NS (ab193972)	1 x 50ml	1 x 50ml
SimpleStep Pre-Coated 96-Well Microplate (ab206978)	1 unit	1 unit
Stop Solution	1 x 12ml	1 x 12ml

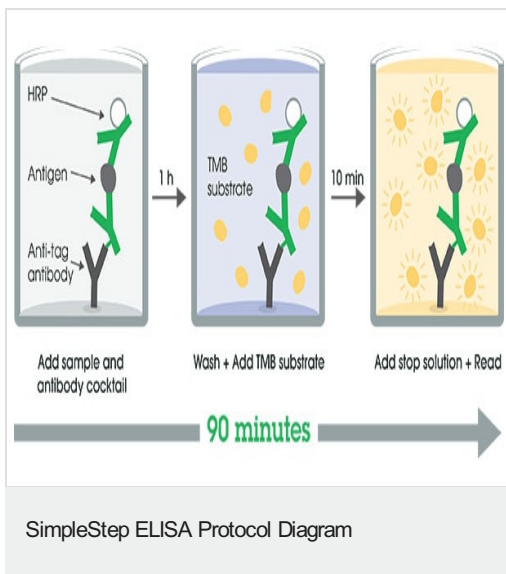
Components	1 x 96 tests	1 x 96 tests
TMB Development Solution	1 x 12ml	1 x 12ml

<b>Function</b>	May be involved in the regulation of dopamine release and transport. Induces fibrillization of microtubule-associated protein tau. Reduces neuronal responsiveness to various apoptotic stimuli, leading to a decreased caspase-3 activation.
<b>Tissue specificity</b>	Expressed principally in brain but is also expressed in low concentrations in all tissues examined except in liver. Concentrated in presynaptic nerve terminals.
<b>Involvement in disease</b>	Genetic alterations of SNCA resulting in aberrant polymerization into fibrils, are associated with several neurodegenerative diseases (synucleinopathies). SNCA fibrillar aggregates represent the major non A-beta component of Alzheimer disease amyloid plaque, and a major component of Lewy body inclusions. They are also found within Lewy body (LB)-like intraneuronal inclusions, glial inclusions and axonal spheroids in neurodegeneration with brain iron accumulation type 1. Parkinson disease 1 Parkinson disease 4 Dementia Lewy body
<b>Sequence similarities</b>	Belongs to the synuclein family.
<b>Domain</b>	The 'non A-beta component of Alzheimer disease amyloid plaque' domain (NAC domain) is involved in fibrils formation. The middle hydrophobic region forms the core of the filaments. The C-terminus may regulate aggregation and determine the diameter of the filaments.
<b>Post-translational modifications</b>	Phosphorylated, predominantly on serine residues. Phosphorylation by CK1 appears to occur on residues distinct from the residue phosphorylated by other kinases. Phosphorylation of Ser-129 is selective and extensive in synucleinopathy lesions. In vitro, phosphorylation at Ser-129 promoted insoluble fibril formation. Phosphorylated on Tyr-125 by a PTK2B-dependent pathway upon osmotic stress. Hallmark lesions of neurodegenerative synucleinopathies contain alpha-synuclein that is modified by nitration of tyrosine residues and possibly by dityrosine cross-linking to generated stable oligomers. Ubiquitinated. The predominant conjugate is the diubiquitinated form. Acetylation at Met-1 seems to be important for proper folding and native oligomeric structure.
<b>Cellular localization</b>	Cytoplasm, cytosol. Membrane. Nucleus. Cell junction, synapse. Secreted. Membrane-bound in dopaminergic neurons.

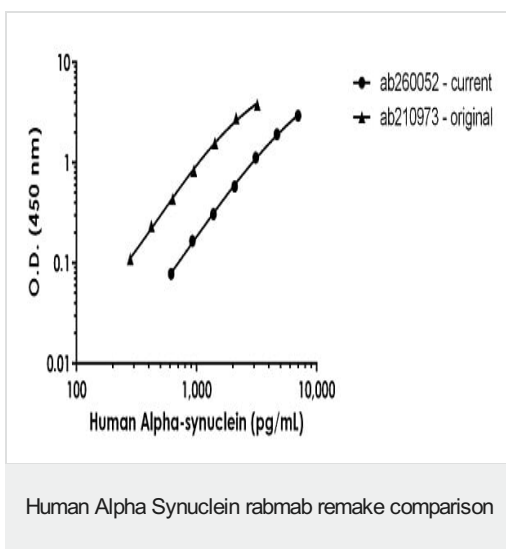
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## Images

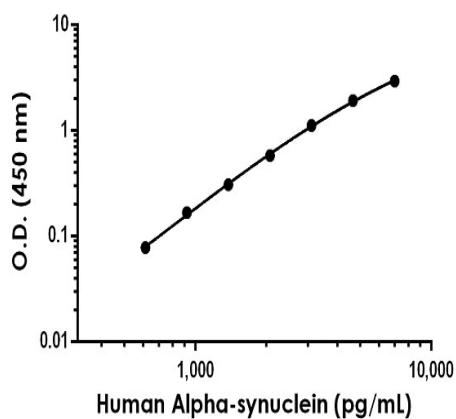
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SimpleStep ELISA technology allows the formation of the antibody-antigen complex in one single step, reducing assay time to 90 minutes. Add samples or standards and antibody mix to wells all at once, incubate, wash, and add your final substrate. See protocol for a detailed step-by-step guide.



Standard Curve comparison between the original Human Alpha-synuclein SimpleStep ELISA kit ([ab210973](#)) and current Human Alpha-synuclein SimpleStep ELISA kit ([ab260052](#)).



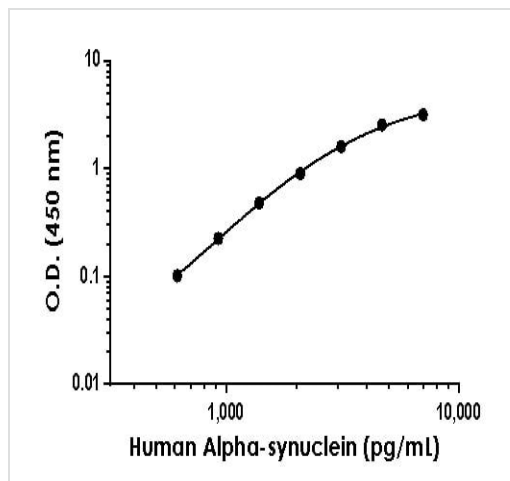
Example of human Alpha-synuclein standard curve in Sample Diluent NS.

The Alpha-synuclein standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean  $\pm$  SD) are graphed.

Standard Curve Measurements			
Concentration (pg/mL)	O.D 450 nm		Mean O.D
	1	2	
0	0.079	0.079	0.079
615	0.161	0.154	0.157
922	0.247	0.244	0.245
1,383	0.392	0.383	0.387
2,074	0.646	0.670	0.658
3,111	1.181	1.221	1.201
4,667	2.081	1.921	2.001
7,000	3.136	2.913	3.025

Example of human Alpha-synuclein standard curve in Sample Diluent NS.

The Alpha-synuclein standard curve was prepared as described. Raw data values are shown in the table. Background-subtracted data values (mean  $\pm$  SD) are graphed.



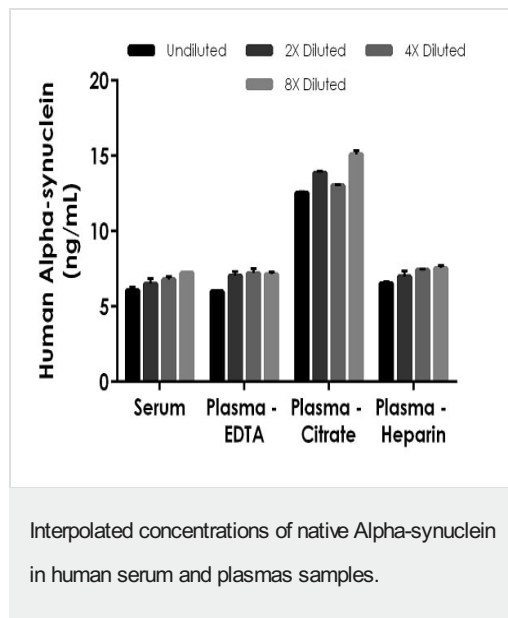
Example of human Alpha-synuclein standard curve  
in 1X Cell Extraction Buffer PTR.

The Alpha-synuclein standard curve was prepared as described.  
Raw data values are shown in the table. Background-subtracted  
data values (mean +/- SD) are graphed

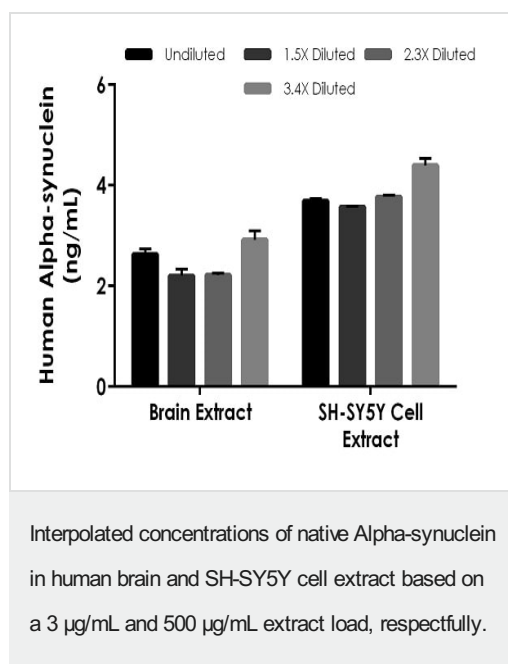
Standard Curve Measurements			
Concentration (pg/mL)	O.D 450 nm		Mean O.D
	1	2	
0	0.057	0.059	0.058
615	0.162	0.158	0.160
922	0.282	0.285	0.284
1,383	0.531	0.544	0.537
2,074	0.943	0.985	0.964
3,111	1.687	1.653	1.670
4,667	2.550	2.663	2.607
7,000	3.192	3.261	3.226

Example of human Alpha-synuclein standard curve  
in 1X Cell Extraction Buffer PTR.

The Alpha-synuclein standard curve was prepared as described.  
Raw data values are shown in the table. Background-subtracted  
data values (mean +/- SD) are graphed.

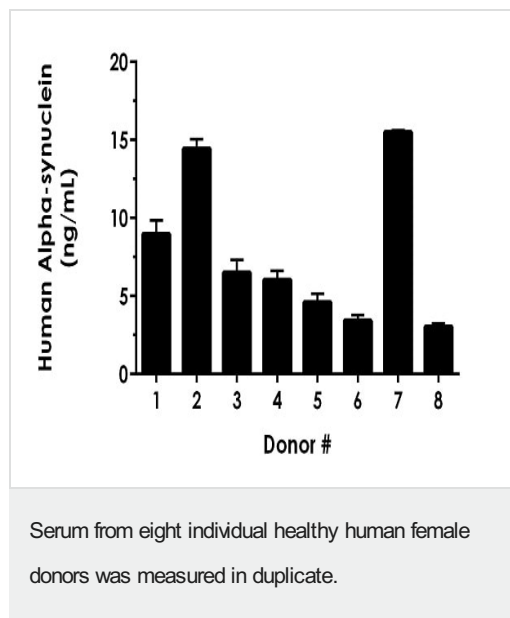


The concentrations of Alpha-synuclein were measured in duplicates, interpolated from the Alpha-synuclein standard curves and corrected for sample dilution. Undiluted samples are as follows: serum 100%, plasma (EDTA) 100%, plasma (citrate) 50% and plasma (heparin) 100%. The interpolated dilution factor corrected values are plotted (mean  $\pm$  SD,  $n=2$ ). The mean Alpha-synuclein concentration was determined to be 6.6 ng/mL in serum, 6.8 ng/mL in plasma (EDTA), 13.6 ng/mL in plasma (citrate), and 7.1 ng/mL in plasma (heparin).

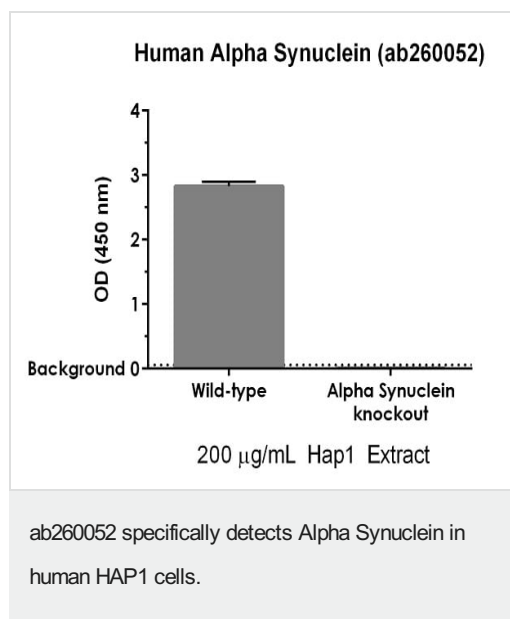


The concentrations of Alpha-synuclein were measured in duplicate and interpolated from the Alpha-synuclein standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean  $\pm$  SD,  $n=2$ ).





Interpolated dilution factor corrected values are plotted (mean  $\pm$  SD,  $n=2$ ). The mean Alpha-synuclein concentration was determined to be 7.8 ng/mL with a range of 3.0 – 8.9 ng/mL.



Raw data is plotted for wild-type and alpha synuclein-knockout HAP1 cell extracts tested at 111  $\mu$ g/mL.

Sample Diluent Buffer	n=	Minimal Detectable Dose
Sample Diluent NS	16	504 pg/mL
1X Cell Extraction Buffer PTR	16	302 pg/mL

Assay sensitivity.

The MDD was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

Dilution Factor	Interpolated value	100% Human Serum	100% Human Plasma (EDTA)	50% Human Plasma (Citrate)	100% Human Plasma (Heparin)
Undiluted	pg/mL	6,075	5,993	6,272	6,541
	% Expected value	100	100	100	100
1.5	pg/mL	3,252	3,524	3,469	3,497
	% Expected value	107	118	111	107
2.3	pg/mL	1,699	1,804	1,629	1,858
	% Expected value	112	120	104	114
3.4	pg/mL	930	893	932	942
	% Expected value	119	119	120	115

Linearity of dilution.

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native Alpha-synuclein was measured in the following biological samples in a 1.5-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	3.0 µg/mL Human Brain Extract	500 µg/mL Human SH-SY5Y cell Extract	50% RPMI 10% FBS
Undiluted	pg/mL	2,633	3,691	3,134
	% Expected value	100	100	100
1.5	pg/mL	1,469	2,381	2,224
	% Expected value	83	97	106
2.3	pg/mL	1,008	1,714	1,509
	% Expected value	84	102	106
3.4	pg/mL	884	1,332	1,002
	% Expected value	111	119	105

Linearity of dilution.

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native Alpha-synuclein was measured in the following biological samples in a 1.5-fold dilution series. Sample dilutions are made in Sample Diluent 1X Cell Extraction Buffer PTR.

Recombinant Alpha-synuclein protein was spiked into RPMI media containing 10% FBS in a 1.5 -fold dilution series. Serial dilutions are made in Sample Diluent NS.

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Sandwich ELISA - Human Alpha-synuclein ELISA  
Kit (ab260052)

To learn more about the advantages of recombinant antibodies see [here](#).

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