BioLegend®

Enabling Legendary Discovery™

LEGEND MAX™

ELISA Kit with Pre-coated Plates

α-Synuclein Aggregate

Cat. No. 449407

ELISA Kit for Accurate Quantitation of
α-Synuclein Aggregates in Cell/Tissue Lysates and
Cerebrospinal Fluid

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biolegend.com

It is highly recommended that this manual be read in its entirety before using this product. Do not use this kit beyond the expiration date.

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Introduction:

α-Synuclein is a 140 amino acid protein involved in the pathology of multiple neurodegenerative diseases, including Parkinson's disease and multiple system atrophy (MSA). This protein localizes to presynaptic terminals in neurons, where it is implicated in regulating neurotransmitter release. α-Synuclein can exist in multiple conformations: a) monomers, b) aggregates (formed from monomers), and c) fibrils (generated from aggregates). These fibrils are the major component of Lewy bodies, which are a hallmark of Parkinson's disease and other dementias. Aggregates of α-synuclein are thought to be toxic, since they can spread to other neurons and can promote additional α-synuclein aggregation and Lewy body formation. Therefore, measurement of α-synuclein aggregates in biological samples is an important tool for neurodegenerative disease research.

The BioLegend LEGEND MAX™ α-Synuclein Aggregate ELISA Kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with rat monoclonal anti-α-synuclein aggregate antibody. The detection antibody is a biotinylated goat polyclonal anti-α-synuclein aggregate antibody. This kit is specifically designed for the accurate quantitation of α-synuclein aggregates in tissue and cell lysates (ex. human brain lysates, transgenic mouse brain lysates) and human cerebrospinal fluid. This kit is analytically validated with ready-to-use reagents.

Materials Provided:

Description	Quantity	Volume (per bottle)	Part #
α-Synuclein Aggregate Pre-coated 96-well Strip Microplate	1 plate		750003002
α-Synuclein Aggregate Detection Antibody	1 bottle	12 mL	750003004
α-Synuclein Aggregate Lyophilized Standard	1 vial	lyophilized	750003006
Avidin-HRP	1 bottle	12 mL	77897
2X Reagent Diluent	1 bottle	32 mL	76457
Wash Buffer (20X)	1 bottle	50 mL	78233
Substrate Solution F	1 bottle	12 mL	79132
Stop Solution	1 bottle	12 mL	79133
Plate Sealers	1 pack		78101

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Materials to be Provided by the End-User:

- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 µL to 1,000 µL
- Deionized water
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer
- Polypropylene vials
- Plate shaker

Storage Information:

Store unopened kit components between 2°C and 8°C. Do not use this kit beyond its expiration date.

Opened or Reconstituted Components	
Microplate wells	If not all microplate strips are used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal. Store between 2°C and 8°C for up to one month.
Standard	The remaining reconstituted standard stock solution can be aliquoted into polypropylene vials and stored at -70°C for up to one month. Avoid repeated freeze-thaw cycles.
Detection Antibody	Store opened reagents between 2°C and 8°C and use within one month.
Avidin-HRP	
2X Reagent Diluent	
Wash Buffer (20X)	
Substrate Solution F	
Stop Solution	

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online at BioLegend's website for details (www.biolegend.com/msds).
2. Substrate Solution F is harmful if inhaled or ingested. Avoid skin, eye, and clothing contact.

3. To reduce the likelihood of transmission of infectious agents, handle all biological samples in accordance with NCCLS regulations.
4. Stop Solution contains strong acid. *Wear eye, hand, and face protection.*
5. Before disposing of the plate, rinse it with an excess amount of tap water.

Specimen Collection and Handling:

The conditions listed below for sample collection and storage are meant to be general guidelines. Sample dilutions may need to be optimized to ensure that they are within the linear range of the ELISA.

Cell/Tissue Lysates: Rinse tissue with PBS, cut it into small pieces (1-2 mm), and homogenize with a tissue homogenizer. For cultured cells, scrape the cells in lysis buffer and transfer to a fresh tube. Lyse the homogenized tissue/cells with an equal amount of lysis buffer for 30 minutes on ice. Agitate the lysate periodically during lysis. Then, centrifuge the lysate to remove any debris. Use the sample immediately; samples can be aliquoted and stored at ≤ -70°C for later use. Repeated freeze-thaw of samples should be avoided.

Reagent and Sample Preparation:

Note: All reagents should be diluted immediately prior to use.

1. Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water. If crystals have formed in the 20X Wash Buffer, bring to room temperature and vortex until dissolved.
2. Reconstitute the α-Synuclein Aggregate Lyophilized Standard by adding the volume of 1X Reagent Diluent to make the standard stock solution (refer to LEGEND MAX Kit Lot-Specific Certificate of Analysis). Allow the reconstituted standard to sit at room temperature for 15-20 minutes, then briefly vortex to mix completely.
3. Initial testing of biological samples should be performed to determine an optimal concentration that is within the linear range of the standard curve. Dilute samples using 1X Reagent Diluent.

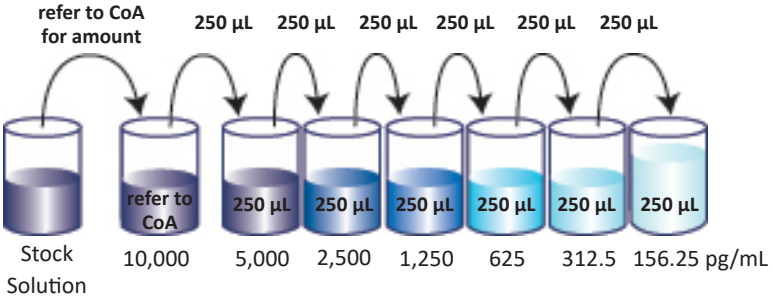
Assay Procedure:

Note: Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this kit.

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.

2. If not all microplate strips will be used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.

3. Prepare 1000 µL of the 10,000 pg/mL top standard by diluting the appropriate amount of the standard stock solution in 1X Reagent Diluent (refer to the LEGEND MAX Kit Lot-Specific Certificate of Analysis). Perform six two-fold serial dilutions of the 10,000 pg/mL top standard in separate tubes using 1X Reagent Diluent as the diluent. Thus, the α-synuclein aggregate standard concentrations in the tubes are 10,000 pg/mL, 5,000 pg/mL, 2,500 pg/mL, 1,250 pg/mL, 625 pg/mL, 312.5 pg/mL and 156.25 pg/mL, respectively. 1X Reagent Diluent serves as the zero standard (0 pg/mL).



4. Wash the plate 4 times with at least 300 µL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping the plate upside down on absorbent paper. All subsequent washes should be performed similarly.

5. Add 50 µL of 1X Reagent Diluent to each well that will contain either standard dilutions or samples.

6. Add 50 µL of standard dilutions or samples to the appropriate wells.

7. Seal the plate with a plate sealer included in the kit and incubate the plate at room temperature for 2 hours with shaking.

8. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.

9. Add 100 µL of α-Synuclein Aggregate Detection Antibody to each well, seal the plate, and incubate at room temperature for 1 hour with shaking.

10. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
11. Add 100 µL of Avidin-HRP to each well, seal the plate, and incubate at room temperature for 30 minutes with shaking.
12. Discard the contents of the plate into a sink, then wash the plate **5 times** with 1X Wash Buffer as in step 4. For this final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
13. Add 100 µL of Substrate Solution F to each well and incubate at room temperature for 15 minutes **in the dark**.* Wells containing α-synuclein aggregates should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.
14. Stop the reaction by adding 100 µL of Stop Solution to each well. The solution color should change from blue to yellow.
15. Read absorbance at 450 nm within 15 minutes. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

*** Optimal substrate incubation time depends on laboratory conditions and the optical linear ranges of ELISA plate readers.**

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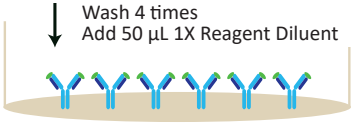
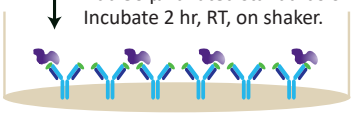
Assay Procedure Summary

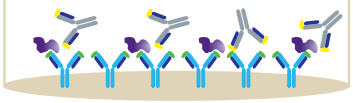
1. Wash 4 times
   Add 50 µL 1X Reagent Diluent



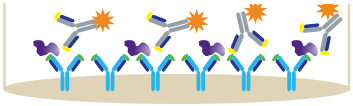
2. Add 50 µL diluted standards or samples.
   Incubate 2 hr, RT, on shaker.



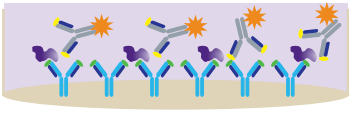
3. Wash 4 times
   Add 100 µL Detection Antibody.
   Incubate 1 hr, RT, on shaker.



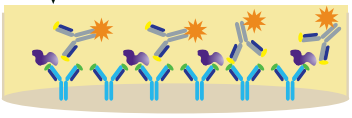
4. Wash 4 times
   Add 100 µL Avidin-HRP.
   Incubate 30 min, RT, on shaker.



5. Wash 5 times.
   Add 100 µL Substrate Solution F.
   Incubate 15 min*, RT, **in the dark**.



6. Add 100 µL Stop Solution.



7. Read absorbance at 450 nm and 570 nm.

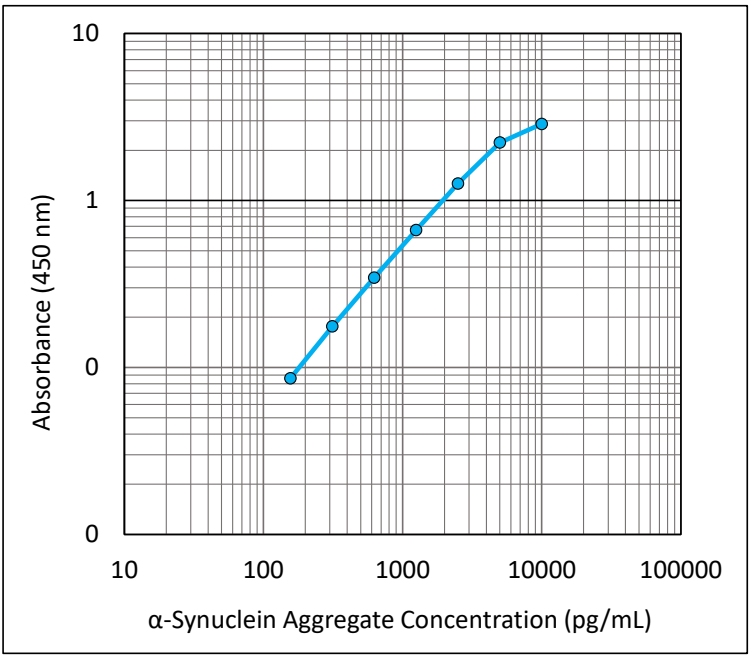
Calculation of Results:

The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If the appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate standards, controls, and samples. Plot the standard curve on log-log graph paper with analyte concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown analyte concentrations, find the mean absorbance value of the unknown concentration on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the concentration.

If samples were diluted, multiply the concentration by the appropriate dilution factor. If a test sample's absorbance value falls outside the linear portion of the standard curve, the test sample needs to be re-analyzed at a higher (or lower) dilution, as appropriate.

Typical Data:

This standard curve was generated at BioLegend for demonstration purposes only. A standard curve must be run with each assay.



Performance Characteristics:

Specificity: No cross-reactivity was observed when this kit was used to analyze mouse α-synuclein monomers at 50 ng/mL. There was approximately 2% cross-reactivity for human α-synuclein monomers at 50 ng/mL. Significant cross-reactivity was observed for mouse α-synuclein pre-formed fibrils, human α-synuclein pre-formed fibrils, and human α-synuclein aggregates at 50 ng/mL.

Sensitivity: The minimum detectable concentration of α-synuclein aggregates is 44.9 ± 18.9 pg/mL.

Recovery: Recombinant mouse α-synuclein aggregates were spiked into several sample types at three different concentrations, then sample recovery was analyzed with the LEGEND MAX™ α-Synuclein Aggregate ELISA kit.

Sample Type	N	Recovery (%)
Mouse Brain Lysate (Human A53T α-Synuclein Transgenic Mice)	1	92.6
Fly Head Lysate (Human α-Synuclein Transgenic Fly)	1	103.2
Normal Human Brain Lysate	1	94.3
Parkinson's Disease Human Brain Lysate	1	96.8
Human Cerebrospinal Fluid	1	79.8
Lysis Buffer	1	83.2

Linearity: The following samples were serially diluted two-fold to produce samples with concentrations within the dynamic range. Then, linearity was analyzed with the LEGEND MAX™ α-Synuclein Aggregate ELISA kit.

Sample Type	N	Linearity (%)
Mouse Brain Lysate (Human A53T α-Synuclein Transgenic Mice)	1	92.8
Fly Head Lysate (Human α-Synuclein Transgenic Fly)	1	83.6
Normal Human Brain Lysate	1	89.6
Parkinson's Disease Human Brain Lysate	1	92.2
Human Cerebrospinal Fluid	1	112.5
Lysis Buffer	1	98.7

Intra-Assay Precision: Sixteen replicates of each of two samples containing different α-synuclein aggregate concentrations were tested in one assay.

Concentration	Sample 1	Sample 2
Number of Replicates	16	16
Mean Concentration (pg/mL)	1350.7	287.9
Standard Deviation	46.6	9.5
%CV	3.5	3.3

Inter-Assay Precision: Two samples containing different concentrations of α-synuclein aggregates were tested in ten independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	10	10
Mean Concentration (pg/mL)	1161.2	265
Standard Deviation	143.8	27.4
%CV	12.4	10.4

Biological Samples: Human A53T α-synuclein-expressing mice were injected with α-synuclein aggregates, and brain lysates were prepared for pons/medulla and whole brain (minus pons/medulla). These samples were generously prepared and provided by Poul Henning Jensen and associates (Dandrite, Aarhus University). Total protein concentrations were quantified via BCA assay, then the lysates were tested using the LEGEND MAX™ α-Synuclein Aggregate ELISA kit. These samples were also tested using the LEGEND MAX™ Human α-Synuclein (Colorimetric) ELISA kit (Cat# 448607) to measure the aggregate-to-total α-synuclein ratio.

Lysates:	Whole brain (no pons/medulla)	Pons/medulla	Pons/medulla
α-Synuclein Aggregates Injected?:	No	No	Yes
α-Synuclein Aggregate (ng/µg Total Protein)	1.97	1.93	1.79
Total α-Synuclein (ng/µg Total Protein)	10.42	10.47	5.86
Aggregate-to-Total α-Synuclein Ratio	0.19	0.18	0.31

Human A53T α-synuclein transgenic mouse brain lysates, human α-synuclein transgenic fly head lysates, and human brain lysates were prepared. Total protein was quantified via BCA assay, then the lysates were tested using the LEGEND MAX™ α-Synuclein Aggregate ELISA kit. Also, human CSF samples were tested.

Sample Type	N	Mean	Min	Max
Transgenic Mouse Brain Lysates (ng/µg Total Protein)	21	2.10	1.42	3.15
Transgenic Fly Head Lysates (ng/µg Total Protein)	3	0.106	0.095	0.118
Human Brain Lysates (ng/µg Total Protein)	6	0.125	0.106	0.153
Human CSF (ng/mL)	4	0.302	ND	0.605

ND = Not detected

Troubleshooting Guide:

Problem	Probable Cause	Solution
High Background	Background wells were contaminated	Avoid cross-well contamination by using the provided plate sealers. Use multichannel pipettes and change tips between pipetting samples and reagents.
	Insufficient washes	Increase number of washes. Increase soaking time between washes prior to addition of substrate solution.
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells.
No or poor signal	Detection Antibody, Avidin-HRP or Substrate solution were NOT added	Rerun the assay and follow the protocol.
	Wrong reagent or reagents were added in wrong sequential order	
	Insufficient plate agitation	The plate should be agitated during all incubation steps using a plate shaker at a speed where solutions in wells are within constant motion without splashing.
	The wash buffer contains Sodium Azide (NaN3)	Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity.
	Incubations were done at an inappropriate temperature, timing or without agitation	Rerun the assay and follow the protocol.
Low or poor standard curve signal	The standard was incorrectly reconstituted or diluted	Adjust the calculations and follow the protocol.
	Standard was inappropriately stored	Store the reconstituted standard stock solution in polypropylene vials at -70°C. Avoid repeated freeze-thaw cycles.
	Reagents added to wells with incorrect concentrations	Check for pipetting errors and the correct reagent volume.

Problem	Probable Cause	Solution
Signal is high, standard curves have saturated signal	Standard reconstituted with less volume than required	Reconstitute new lyophilized standard with the correct volume of solution recommended in the protocol.
	Standards/samples, detection antibody, Avidin-HRP or substrate solution were incubated for too long	Rerun the assay and follow the protocol.
Sample readings are out of range	Samples contain no or below detectable levels of the analyte	If samples are below detectable levels, it may be possible to use a larger sample volume. Contact technical support for appropriate protocol modifications.
	Samples contain analyte concentrations greater than highest standard point	Samples may require dilution and analysis.
High variation in samples and/or standards	Multichannel pipette errors	Confirm that pipette calibrations are accurate.
	Plate washing was not adequate or uniform	Ensure pipette tips are tightly secured. Ensure uniformity in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before assaying.
	Samples may have high particulate matter	Remove particulate matter by centrifugation.
	Cross-well contamination	Do not reuse plate sealers. Always change tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.

ELISA Plate Template

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Legend Max™ Kits are manufactured by BioLegend Inc.
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