SensoLyte® Anti-alpha-Synuclein Quantitative ELISA Kit (Rat)
Colorimetric

Revision number: 1.3 *Last updated: 1/15/18*

Catalog #	AS-55550-R
Kit Size	One 96-well strip plate

This kit is optimized to detect rat alpha-Synuclein (α-Synuclein) in cell and tissue lysate, and in body fluids such as plasma. Wells are pre-coated with anti-α-Synuclein monoclonal antibodies, blocked, and stabilized for long-term storage. The amount of α-Synuclein is quantified using sandwich ELISA: rabbit polyclonal anti-α-Synuclein specific antibodies directly conjugated to horseradish peroxidase (HRP) are used to detect captured α-Synuclein.

- ***One Step Convenient Format***
  - Pre-coated and pre-blocked 96-well strip plate
  - Ready-to-use substrate solution and other assay components
  - One step assay (samples and detection antibody are added simultaneously)
  - 1 hour assay time at room temperature (excluding incubation time)
- ***Minimal Sample Size***
  - Requires only 10-20 µl of cell and tissue lysate or body fluids
- ***High Sensitivity***
  - Detects 5 pg/ml of α-Synuclein (calculated as three standard deviations from the blank)
- ***Broad Dynamic Range***
  - 8-500 pg α-Synuclein/ml in the assayed sample

Kit Components and Handling

Component	Description	Quantity
Component A	Anti-α-Synuclein 8-well strip plate (8x12)	12 x 8 well strips
Component B	α-Synuclein Standard, Rat (1 µg/vial)	3 vials
Component C	1X Sample Dilution Buffer	30 ml
Component D	10X Wash Buffer	30 ml
Component E	TMB Color Substrate Solution	10 ml
Component F	Stop Solution	10 ml
Component G	Detection Antibody Rabbit Polyclonal anti-rat-α-Synuclein IgG-HRP (10 µg/50µl)	50 µl
Component H	Adhesive Plate Covers	2 pieces

Other Materials Required (but not provided)

- Microplate reader: Capable of reading absorbance at 450 nm
- Rocking platform or shaker
- Protease inhibitor such as AEBSF [4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride]
- Computer software capable of plotting linear regression curves
- Plate washer (optional)

Kit is shipped on blue ice. Store all kit components at 2-4°C for up to 6 months.

For Research Use only

Introduction

α-Synuclein is a major component of Lewy bodies in the affected neurons in Parkinson's disease[1-4]. This protein has a mass of 14.5 kDa (140 amino acids long) and consists of a conserved degenerative amino-terminal domain and an acidic carboxyl-terminal with higher sequence divergence[1-4]. α-Synuclein is predominantly expressed in brain: specifically in cerebellum, thalamus, neocortex, hippocampus, and striatum regions[1-2]. Other tissues express α-Synuclein at very low levels[1-4]. The physiological role of α-Synuclein is not yet well understood. However, the presence of imperfect KTKEGV lipid interacting repeats suggests that it may be involved in synaptic vesicle homeostasis[3].

The SensoLyte® α-Synuclein Quantitative ELISA Kit (Rat) provides a convenient and quantitative assay for determining α-Synuclein amount in cell and tissue lysate as well as in body fluids. Compared to other anti-α-Synuclein ELISA kits on the market, it takes less time to run this assay. HRP conjugated detection antibody in this kit is added simultaneously with samples and standards during the assay. This eliminates extra incubation and washing steps and makes this kit one-step procedure for α-Synuclein quantification.

Experimental Protocol

Please Note:

a) Bring all kit components to room temperature before starting the assay
b) Spin down all components before use
c) Mix well the 10X Washing Buffer to dissolve any precipitated salt before diluting with water
d) Standard must be diluted in the same buffer composition as samples tested. For example, if assaying RIPA brain lysate sample diluted at 1:10 ratio with "Sample Dilution Buffer", standards must be diluted in "Sample Dilution Buffer" with 10% RIPA final concentration to ensure consistent and accurate results.

1. ELISA assay

1.1 Reconstitute rat α-Synuclein Standard (Component B) with 1 ml of Sample Dilution Buffer (Component C) or Sample Dilution Buffer mixed with lysis solution (see Note "d"). Mix gently (do not vortex) and let stand for 10-15 minutes. Reconstituted standard must be used within one hour to ensure accurate results. **Do not re-use the reconstituted Standard!**

1.2 Arrange and label strips (Component A) based on the number of wells for standard and samples. Although diluted standard and samples can be run as single points, duplicates are recommended. Instructions for preparing cell and/or tissue lysates are provided in the Appendix.
Place unused strips into the plate bag and seal completely.

1.3 Make serial dilution of rat α-Synuclein Standard (Component B) with Sample Dilution Buffer (Component C). Refer to Table 1.

Table 1. Serial dilution of the α-Synuclein Standard.

Step	Concentration [pg/ml]	Rat α-Synuclein Standard (Component B)	Sample Dilution Buffer (Component C)
1	1000000	*Prepare as described in Step 1.1*	
2	10000	10 µl from step 1	990 µl
3	**500**	50 µl from step 2	950 µl
4	**250**	500 µl from step 3	500 µl
5	**125**	500 µl from step 4	500 µl
6	**62.5**	500 µl from step 5	500 µl
7	**31.25**	500 µl from step 6	500 µl
8	**15.625**	500 µl from step 7	500 µl
9	**7.8125**	500 µl from step 8	500 µl

1.4 Dilute Detection Antibody (Component G) 100 fold in Sample Dilution Buffer (Component C). Prepare 50 µl of the above for each well to be run in the assay.

1.5 Add 100 µl of the diluted standards in duplicates including blank (Start with Step 3 from Table 1). We recommend to dilute rat plasma at 1:50 ratio with Sample Dilution Buffer to avoid sample matrix effect. In addition, protease inhibitor AEBSF should be added to all samples at 1mM final concentration to avoid protein degradation.

1.6 Add 100 µl per well of the diluted samples into appropriate wells (depending on the number of samples to be tested).

1.7 Add 50 µl of the diluted Detection Antibodies (from step 1.4) into each well to be tested, cover the plate with Adhesive Plate Cover (Component H), and incubate it at room temperature for 4 hours or 4 °C overnight. **Protect plate from direct light!**

1.8 Prepare 1X working wash buffer by diluting the 10X Wash Buffer (Component D) with deionized H_2O.

1.9 After plate incubation, aspirate the wells and wash them with 350 µl/well of 1x Wash Buffer 6 times. Allow 5-10 seconds lag time before emptying the wells between washes. Pat dry the plate using a paper towel and clean outside of wells with non-abrasive paper to ensure accurate optical reading.

1.10 Add 100 µl of the TMB color substrate solution (Component E) into each well. Incubate plate at room temperature until blue gradient is clearly observed across the wells (5-15 minutes). It may be necessary to adjust color development time so that absorbance values fall within the detection range. Samples may require further dilution if α-Synuclein concentration is too high.

1.11 Add 100 µl of the Stop Solution (Component F) into each well (blue color will turn to yellow). Measure absorbance (OD) at 450 nm using a microplate absorbance reader within 20 minutes after adding the Stop Solution.

2. Calculate the concentration of α-Synuclein in samples.

2.1 Determine the average values (if replicates are used) for the standard and sample absorbance readings. Plot calibration curve using linear regression curve-fit and determine linear equation for the concentration-absorbance relation. R^2 should be higher than 0.98. There should be at least 5 serially diluted standard concentrations in the calculation to ensure statistical significance.

2.2 Choose absorbance values for the diluted samples that are within the range used in the standard curve and calculate the concentration of α-Synuclein in the sample(s). Calculated concentrations must be multiplied by the sample dilution factor.

2.3 Typical α-Synuclein Standard Curve:

Please note, new standard curve must be generated each time the assay is run.

Table 2. An example of an α-Synuclein ELISA standard curve.

alpha-Synuclein, pg/ml	OD @ 450 nm, alpha-Synuclein
500	2.754
250	1.467
125	0.857
62.5	0.421
31.25	0.246
15.625	0.101
7.8125	0.074
0	0.000

Note: *Standards were run in triplicates; blank value was subtracted from the absorbance readings.*

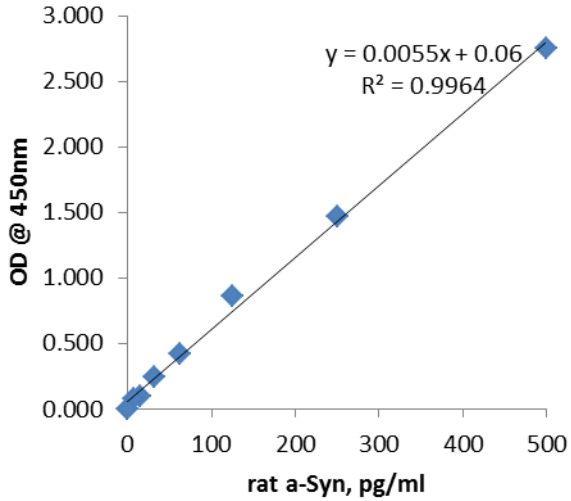

Figure 1. An example of Rat α-Synuclein ELISA standard curve.

3. Kit Performance.

3.1 Rat α-Synuclein Recovery Assay:

Rat α-Synuclein was added to the diluted rat plasma at 0pg/ml, 20pg/ml or 200pg/ml and assayed using the kit ten times.

Specimen	Theoretical (spiked) Value, pg/ml	Measured Value, pg/ml	% Recovery
Rat Plasma +0pg/ml a-Syn	N/A	5.8*	N/A
Rat Plasma +20pg/ml a-Syn	20	22.54	112
Rat Plasma +200pg/ml a-Syn	200	191	95.5

**Diluted rat plasma has measureable α-Synuclein*
Plasma was diluted 50 times with Sample Dilution Buffer (Component C)
Blank Plasma readings were subtracted prior to the calculations

3.2 Intra-Assay Variation Test:

Measurement Value, pg/ml	Standard Deviation	Coefficient of Variation	n
20	0.014	6.1	24

***n**-number of replicates*

Appendix

A) Buffer composition for brain homogenate:

5M Guanidine HCl
50 mM Tris HCl, pH=8.0

Mix 800 µl of the above solution with 100 mg of brain sample in a Dounce homogenizer placed on ice. Homogenize the tissue thoroughly and incubate at room temperature for 3-4 hours.
Dilute brain homogenate with "Sample Dilution Buffer" for the assay. We recommend 1:1000 dilution to start with for mouse brain homogenate.

B) RIPA Buffer for Cell and Tissue Lysate
(150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 50 mM Tris Base, 5mM EDTA, 1mM EGTA)

NaCl	877 mg
DOC	50 mg
SDS	10 mg
Tris-Cl	606 mg
Deionized H_2O to	99 ml
Adjust pH to 8.0 with NaOH	
EDTA	146 mg
EGTA	38 mg
Mix vigorously	
Triton X-100	1 ml

Lysates must be diluted with "Sample Dilution Buffer" at least 1:50 ratio. Optimal dilution ratio depends on the amount of α-Synuclein present.

References:

1. Rivers, R. et al. *Protein Science* **17**, 887-898 (2008).
2. Bruening, W. et al. *Cancer.* **88**, 9, 2154-2163 (2000).
3. George M. J. *Genome Biology* **3**, 1, 1-6 (2001).
4. Latawiec, D. et al. *PloS ONE* **5**, 2, 1-8 (2010).