

# Human Alpha-synuclein ELISA Kit

## Sandwich ELISA

### Catalog EL-213-015

#### INTRODUCTION

Alpha-synuclein, encoded by SNCA, is an abundant presynaptic protein concentrated at neuronal nerve terminals, where it helps orchestrate neurotransmitter release. As a free monomer it assists synaptic-vesicle priming and helps widen the fusion pore during exocytosis, partly through local calcium handling, while in its membrane-associated multimeric form it acts as a chaperone that helps SNARE proteins assemble correctly for vesicle docking. It also shapes dopaminergic signaling through an interaction with the dopamine transporter, and has been reported to associate with tau, connecting it to cytoskeletal regulation of vesicle movement. Mutations in SNCA cause autosomal-dominant forms of Parkinson disease and dementia with Lewy bodies, and fragments of the protein are also detected within the amyloid plaques characteristic of Alzheimer disease brain pathology.

#### PRINCIPLE OF THE ASSAY

This kit uses a sandwich enzyme immunoassay format. The microtiter plate wells are pre-coated with a capture antibody specific to human Alpha-synuclein (SNCa). Standards or samples are added to the appropriate wells along with a biotin-conjugated detection antibody that also recognizes human SNCa. Avidin conjugated to horseradish peroxidase (HRP) is then added to each well and allowed to incubate. Upon addition of TMB substrate solution, a colorimetric signal develops only in wells where human SNCa, the biotin-conjugated antibody, and the avidin-HRP conjugate are all present. The enzymatic reaction is stopped by the addition of a sulphuric acid stop solution, and the resulting color change is measured spectrophotometrically at 450 nm ( $\pm 10$  nm). Sample concentrations are determined by comparing the optical density of each sample against a standard curve generated from the kit standards.

#### MATERIALS AND STORAGE

Store kit components at  $-20^{\circ}\text{C}$  unless specified otherwise. DO NOT USE past kit expiration date. Some vials contain a small amount of reagents. Spin tubes on pulse setting prior to opening.

| Each kit includes:                                                   | Units               |
|----------------------------------------------------------------------|---------------------|
| Pre-Coated Microplate                                                | 12 strips x 8 wells |
| Standard (Lyophilized)                                               | 2 vials             |
| Biotinylated Antibody (100 $\times$ )                                | 120 $\mu\text{L}$   |
| Streptavidin-HRP (100 $\times$ )                                     | 120 $\mu\text{L}$   |
| Standard/Sample Diluent Buffer                                       | 20 mL               |
| Biotinylated Antibody Diluent                                        | 12 mL               |
| HRP Diluent                                                          | 12 mL               |
| Wash Buffer (25 $\times$ )                                           | 20 mL               |
| TMB Substrate Solution                                               | 10 mL               |
| Stop Reagent                                                         | 6 mL                |
| Plate Covers                                                         | 2 pieces            |
| <i>Do not mix or substitute reagents with those from other lots.</i> |                     |

#### MATERIALS AND INSTRUMENTS REQUIRED BUT NOT SUPPLIED

- Precision pipettes calibrated to deliver 5-1000 $\mu\text{L}$
- Multi-channel pipette calibrated to deliver 50-200 $\mu\text{L}$
- Plate shaker
- Disposable tips
- Vortex-Mixer
- Distilled or de-ionized water
- Microplate reader capable of reading 450nm with background subtraction at 620nm

#### SAFETY PRECAUTIONS

- The test protocol must be followed strictly.
- All reagents containing human material should be handled as if potentially infectious. Operators

should wear gloves and protective clothing when handling any patient sera or serum based products.

- The kit reagents contain antimicrobial agents, acid and 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
- Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local regulations.
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- Only trained laboratory personnel should execute this test.

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#### PREPARATION OF REAGENTS

Prepare only the appropriate amount of required reagent on the day of use. Store all reagents as per instructions stated on the label.

- 1. Wash Buffer (1X) Preparation: Dilute the 25X wash buffer concentrate 1:25 with double-distilled Water.**
- 2. Detection Reagent (1X) Preparation: Dilute the biotinylated antibody and streptavidin-HRP concentrates 100-fold with Biotinylated Antibody Diluent and HRP Diluent, respectively, immediately before use.**
- 3. Preparation of Calibrators: Prepare calibrators by serial dilution from a 1000 down to 15.63 (plus a 0 blank), per the source manual.**

| Sol'n ID | Source         | Source Vol (μL) | Cal. Diluent (μL) | Final Vol (μL) | Final Conc. (ng/mL) |
|----------|----------------|-----------------|-------------------|----------------|---------------------|
| Std 1    | Stock Standard | 500             | 500               | 1000           | 1000                |
| Std 2    | Std 1          | 500             | 500               | 1000           | 500                 |
| Std 3    | Std 2          | 500             | 500               | 1000           | 250                 |
| Std 4    | Std 3          | 500             | 500               | 1000           | 125                 |
| Std 5    | Std 4          | 500             | 500               | 1000           | 62.5                |
| Std 6    | Std 5          | 500             | 500               | 1000           | 31.25               |
| Std 7    | Std 6          | 500             | 500               | 1000           | 15.63               |
| Blank    | Std 7          | 500             | 500               | 1000           | 0                   |

#### SPECIMEN STORAGE

This kit is compatible with EDTA-plasma, heparin-plasma and serum samples. Samples can be stored at or below -20°C for up to 1 year.

#### ASSAY PROCEDURE

1. After the kit is equilibrated at room temperature, add 100 μL of Standard Working Buffer (gradually diluted according to the instructions) or 100 μL of sample to each well, and incubate at 37°C for 80 minutes.
2. Discard the liquid in the plate, add 200 μL 1× Wash Buffer to each well, and wash the plate 3 times. After pat it dry against clean absorbent paper, add 100 μL Biotinylated Antibody Working Solution (1×) to each well, incubate at 37°C for 50 minutes.
3. Discard the liquid in the plate, add 200 μL 1× Wash Buffer to each well, and wash the plate 3 times. After pat it dry against clean absorbent paper, add 100 μL 1× Streptavidin-HRP Working Solution to each well, incubate at 37°C for 50 minutes.
4. Discard the liquid in the plate, add 200 μL 1× Wash Buffer to each well, and wash the plate 5 times. After pat it dry against clean absorbent paper, add 90 μL TMB Substrate Solution to each well, incubate at 37°C for 20 minutes in the dark.
5. Add 50 μL Stop Solution to each well, shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm immediately, calculation of the results.

#### CALCULATIONS AND RESULTS

1. Construct a standard curve by plotting the absorbance obtained from each standard against concentration. Use a 4 or 5 parameter curve fit. Alternatively a log-log curve fit may be used.
2. The concentration of the unknowns can be read directly from this standard curve using the absorbance value for each

sample.

3. Any sample undiluted or diluted still reading greater than the highest standard should be diluted appropriately and retested. If the samples have been diluted, the concentration determined from the standard curve must be multiplied by the dilution factor.

#### **PERFORMANCE CHARACTERISTICS**

**Precision: Intra-assay CV < 8%. Inter-assay CV < 10%.**

**Detection Limit: 6.7 pg/mL**

**Recovery: Serum (n = 5): 80-95% (avg 87%); EDTA plasma (n = 5): 92-105% (avg 98%); Heparin plasma (n = 5): 84-96% (avg 90%)**

**Specificity: This assay has high sensitivity and excellent specificity for detection of Human SNCa. No significant cross-reactivity or interference between Human SNCa and analogues was observed.**



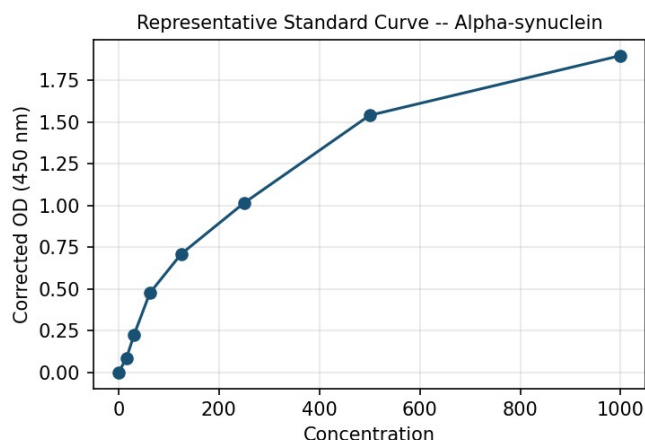
**This kit employs a sandwich enzyme immunoassay to quantify Alpha-synuclein (SNCa) in biological samples.**

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#### **SAMPLE STANDARD CURVE**



Representative standard curve shown for illustration only. This curve should not be used to calculate unknown sample concentrations -- run a new standard curve with each assay.

#### **ORDERING INFORMATION**

Please visit [www.affinityimmuno.com](http://www.affinityimmuno.com) to order this product. Visa, Mastercard, AMEX and PayPal are accepted in our online store.

Your order will be processed immediately and you will be notified with a delivery timeframe.