Human Alpha-Synuclein (SNCA) ELISA Protocol

FIVEphoton Biochemicals

For research use only.
Not for diagnostics.

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Store: 4°C
Safety: Stop solution contains acid. Avoid all contact and inhalation.

Human Alpha-Synuclein (SNCA) ELISA Kit

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Storage: 4°C, six months after manufacture. Longer term storage at -20°C of ELISA strip plate (and sections), standard peptide and other solutions.

Standard Peptide Concentration: 135pg/ml

Assay Range: 3.0pg/m – 135pg/ml

This ELISA kit is based on the double-antibody sandwich technique to detect human α-synuclein. The kit detects soluble α-synuclein from a variety of sources, including biological fluids, cell and tissue homogenates and lysates. It can be used to measure human α-synuclein expressed in transgenic mouse models as well as in recombinant cell culture systems. Additionally, Triton-based cell lysis buffers can be employed to extract α-synuclein samples from the cytoplasmic (and membranous) locations of cells that can be assayed by this kit.

Experimental Principles

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay human α-synuclein concentration. Samples are applied to microelisa wells pre-coated with an affinity purified anti- α-synuclein polyclonal antibody. Samples are incubated and then washed. A second polyclonal anti-α-synuclein conjugated antibody with HRP is then added, followed by incubation and wash. Chromogen solutions A and B are added resulting in a color change to blue. A stop solution is applied to terminate the reaction, turning the solution to yellow. Absorbance readings at 450 nm corresponding to that of the provided standard solution are used to estimate α-synuclein concentration in the samples.

Materials supplied. Store all materials at 4°C

1	Standard peptide: 135pg/ml	0.5ml	7	Chromogen Solution A	6ml
2	Standard diluent	1.5 ml	8	Chromogen Solution B	6ml
3	Microelisa Strip plate	12 well×8strips	9	Stop Solution	6ml
4	HRP-Conjugate Antibody	6 ml	10	Instruction Manual	1
5	30×wash solution	20ml	11	Closure plate membrane	2
6	Sample diluent	6ml	12	Sealed bags	1

Materials required but not supplied

1. 37°C incubator
2. Standard absorbance microplate reader
3. Precision pipettes and disposable pipette tips
4. De-ionized water
5. Disposable tubes for sample dilution
6. Absorbent paper

Important notes and preparation for the assay

1. The kit should be equilibrated to room temperate for 30 min prior to performing the assay. Store opened microelisa plates in a sealed plastic bag at 4°C, or at -20°C for longer term storage. Avoid multiple freeze-thaws. A multi-channel sampler that has been calibrated for accuracy is a preferred method to apply samples. Plates should be sealed during the assay. Wells should not be allowed to dry. Limit light exposure to the chromogen solutions.
2. Perform dilution of the standards, blanks and experimental samples in a separate 96-well plate, not in the ELISA wells. Transfer solutions simultaneously to the ELISA dish.
3. The experimenter should perform preliminary tests to identify the sample dilution required to meet the assay range. Perform a preliminary assay with your samples, using standards at the high and low dilution. Suspend and dilute your experimental samples into the "sample diluent" provided with this kit to meet the requirements of the assay. A dilution series of several samples may be required to identify the correct concentration that meets the assay range. Concentrate or dilute your samples if adjustment is needed. Set aside sufficient experimental sample for reserve to repeat the assay.
4. Determine whether your sample vehicle reacts with the assay: Dilute your sample in the provided sample diluent or prepare samples in another vehicle to prevent inadvertent experimental readings.
5. It is recommended that samples are assayed in duplicate to address pipetting error.
6. Use new applicator tips and ELISA plate sealants to avoid cross-contamination.
7. Do not mix reagents from other ELISA kits.
8. Note that sodium azide in samples that is not washed away may inhibit HRP.
9. When calculating concentration of your sample from the assay, make sure to take into account the dilution factor.
10. If wash solution crystallizes during storage at 4°C, heat solution at 37°C and shake until crystals suspend.

Assay procedures

Standard and Sample Preparation. Standards and Samples should be added simultaneously to wells. Prepare the standards and samples in a separate 96-well dish and transfer simultaneously to the ELISA dish.

Assay procedure

1. Set aside and mark 14 wells for standard peptide dilutions. Configure seven concentrations of standard peptide in duplicate, suspended and mixed as indicated below in a separate 96-well dish. Do not use the ELISA wells directly to perform the dilutions: there should be 7 wells in duplicate. The final total volume in each well should be 50µl.

Table 2. Standard dilutions

135pg/ml	Standard No. 7	50µl standard Peptide
90pg/ml	Standard No.6	100µl standard peptide + 50µl standard diluent, mix. Remove 100µl to make standard 5.
60pg/ml	Standard No.5	100µl Standard No. 6 + 50µl standard diluent, mix. Remove 100µl to make standard 4.
30pg/ml	Standard No.4	100µl Standard No. 5 + 100µl Standard diluent, mix. Remove 50µl to make standard 3. Remove and discard 100µl.
15pg/ml	Standard No.3	50µl Standard No.4 + 50µl Standard diluent, mix. Remove 50µl to make standard 2.

7.5pg/ml	Standard No.2	50µl Standard No.3 + 50µl Standard diluent, mix. Remove 50µl to make standard 1.
3.25pg/ml	Standard No. 1	50µl of standard 2 + 50µl Standard diluents, mix. Discard 50µl.

2. Set up 2 wells as blanks. In the blank wells, add 40µl sample diluent and 10µl sample vehicle. For the blank wells, perform all procedures of the assay, except do not add HRP conjugated antibody.

3. For wells with experimental samples, add 40µl sample diluent in each well, then add 10µl of your samples. This creates a 5X dilution factor of sample which should be accounted for later when calculating sample concentration.

4. Transfer the standard solutions, blank well solutions and experimental samples simultaneously to the ELISA dish.

5. Use the closure membrane to enclose the plate, mix gently with a rotating table, and incubate for 30 min at 37^{o}C, or 1 hr at RT.

6. Dilute the 30X Wash Solution with dH_20. Make 3 ml of diluted wash solution for each assay well.

7. After the first incubation period, discard the liquid in the wells by gently aspirating. Turn the plate upside down and gently pat dry the plate with an absorbent paper. To wash the wells, fill each well with 100 µl of diluted Wash Solution, oscillate gently with the rocker table for 2 min, and then aspirate off the liquid. Pat dry the microplate with absorbent paper. Repeat the wash steps 5 times except for 30 sec each time.

8. Simultaneously transfer 50 µl HRP-conjugate reagent to each well, except for the blank wells. Rotate gently for 1 hr at 37^{o}C for 30 min or 1 hr at RT.

9. Wash the wells as described in Step 7 above. Remove the liquid from the wells.

10. Prepare a solution of 50 µl chromogen solution A and 50µl chromogen solution B for each well. Transfer the chromogen solutions simultaneously to the wells. Gently mix for 15 min at 37^{o}C in the dark. Limit light exposure to the chromogen solutions.

11. Simultaneously add 50 µl of Stop Solution to each well. Upon addition of stop solution, the blue color should immediately change to yellow.

12. Measure the optimal density (OD) at 450 nm within 15 minutes of adding the stop solution. Set the blank wells as zero.

13. If sample readings are higher than the most concentrated standard, you may wish to dilute the samples and assay again.

Data Analysis

1. Compile a standard curve using the blank standard solutions and the corresponding OD values. You may wish to calculate a linear regression equation from the standard curve to determine the concentration of your samples. Remember that samples were diluted 5 fold in the assay in your final calculation. Other forms of analysis can also be applied to estimate the concentration of your samples.

Flow chart of the procedures

Prepare standards, blank and samples

Add samples to wells, incubate for 1 hr at RT or 30 min at 37^{o}C.

Wash each well five times.

Add HRP conjugate antibody to each well, incubate for 1 hr at RT or 30 min at 37^{o}C.

Wash each well five times

Add chromogen solutions A and B, 15 min at 37^{o}C

Add stop solution

Measure OD 450 nm within 15 min