研究用試薬

Alpha-Synuclein ELISA
Instructions for use

ORDER NO.	ANTIGEN	SUBSTRATE	FORMAT
EQ 6545-9601-L	Alpha-synuclein	Ab-coated microplate wells	96 x 01 (96)

Principles of the test: The test kit contains microplate strips each with 8 break-off reagent wells coated with monoclonal anti-alpha-synuclein antibodies. In the first analysis step, the calibrators and samples are diluted with biotin-labelled anti-alpha-synuclein antibodies (biotin) and added to the microplate. In this process, alpha-synuclein is bound in a complex. To detect these complexes, a second incubation is carried out using a peroxidase-labelled streptavidin (enzyme conjugate). A following incubation with substrate and chromogen promotes a colour reaction. The colour intensity is proportional to the alpha-synuclein concentration in the sample.

Contents of the test kit:

Component	Colour	Format	Symbol
1. Antibody-coated microplate wells 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	---	12 x 8	STRIPS
2. Calibrator 1, alpha-synuclein, lyophilised	colourless	1 x 500 µl	CAL 1
3. Calibrator 2, alpha-synuclein, lyophilised	colourless	1 x 500 µl	CAL 2
4. Calibrator 3, alpha-synuclein, lyophilised	colourless	1 x 500 µl	CAL 3
5. Calibrator 4, alpha-synuclein, lyophilised	colourless	1 x 500 µl	CAL 4
6. Calibrator 5, alpha-synuclein, lyophilised	colourless	1 x 500 µl	CAL 5
7. Calibrator 6, alpha-synuclein, lyophilised	colourless	1 x 500 µl	CAL 6
8. Calibrator 7, alpha-synuclein, lyophilised	colourless	1 x 500 µl	CAL 7
9. Control 1, alpha-synuclein, lyophilised	colourless	1 x 500 µl	CONTROL 1
10. Control 2, alpha-synuclein, lyophilised	colourless	1 x 500 µl	CONTROL 2
11. Biotin, ready for use	green	1 x 12 ml	BIOTIN
12. Enzyme conjugate, ready for use	blue	1 x 12 ml	CONJUGATE
13. Wash buffer 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
14. Chromogen/substrate solution TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
15. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
16. Test instruction	---	1 booklet	
17. Quality control certificate	---	1 protocol	

LOT Lot description

For research use only

Storage temperature

Unopened usable until

Storage and stability: The test kit has to be stored at a temperature between +2 °C and +8 °C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Modifications to the former version are marked in grey.

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18 °C to +25 °C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2 °C to +8 °C and protected from contamination, unless stated otherwise below.

- **Coated wells:** Lot-specific, ready for use. Do not use the plate if the protective wrapping is damaged. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
  Once the protective wrapping has been opened for the first time, the wells coated with antibodies can be stored in a dry place and at a temperature between +2 °C and +8 °C for 4 months.

- **Calibrators and controls:** Lot-specific. Reconstitute calibrators and controls with 500 µl deionised or distilled water approximately 10 minutes before use and mix thoroughly upside down. Before use, confirm that the lyophilisate is completely dissolved in the water. Reconstituted calibrators and controls are ready for use and must be frozen at -20 °C immediately after use. Calibrators and controls can undergo three freeze/thaw cycles. Minimise storage at room temperature at all costs. Mix thoroughly before use.

- **Biotin:** Lot-specific, ready for use. Biotin-labelled alpha-synuclein detection antibody. Mix thoroughly before use.

- **Enzyme conjugate:** Lot-specific, ready for use. Peroxidase-labelled streptavidin. Mix thoroughly before use.

- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to +37 °C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionised or distilled water (1 part reagent plus 9 parts distilled water).
  For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
  The working-strength wash buffer is stable for 4 weeks when stored at +2 °C to +8 °C and handled properly.

- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue-coloured.

- **Stop solution:** Ready for use.

Warning: Some of the reagents contain preserving agents in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the samples

Samples: Cerebrospinal fluid (CSF).

Notes on sample handling: It is of particular importance to sample CSF directly into polypropylene tubes upon lumbar puncture. If the analysis is not to be performed immediately following sampling, the samples should be stored at -80 °C and subjected to preferably no more than one, if necessary two, freeze/thaw cycles. Standardisation of pre-analytical sample treatment is a prerequisite of accurate Alzheimer's-specific CSF testing. In this context, also consider the recommendations of the Alzheimer's Biomarkers Standardisation Initiative (ABSI).

Incubation

Sample incubation:
(1st step)

Pipette **100 µl** of biotin solution and **25 µl** of calibrators, controls or undiluted samples into each of the reagent wells.
Incubate for **180 min** at room temperature (+18 °C to +25 °C).

Washing:

Manual: Empty the wells and subsequently wash 5 times using 300 µl of working strength wash buffer for each wash.
Automatic: Remove the protective foil and wash the reagent wells 5 times with 450 µl of working-strength wash buffer (program setting: e.g. TECAN Columbus Washer “Overflow Mode”).

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (> 10 µl) in the reagent wells after washing can interfere with the substrate and lead to false low extinction readings.
Insufficient washing (e.g. less than 5 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction readings.
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Enzyme conjugate incubation:
(2nd step)

Pipette **100 µl** of enzyme conjugate (streptavidin-peroxidase) into each of the microplate wells and incubate for **30 minutes** at room temperature (+18 °C to +25 °C).

Washing:

Empty the wells. Wash as described above.

Substrate incubation:
(3rd step)

Pipette **100 µl** of chromogen/substrate solution into each of the microplate wells. Incubate for **30 minutes** at room temperature (+18 °C to +25 °C), protect from direct sunlight.

Stopping:

Pipette **100 µl** of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:

Photometric measurement of the colour intensity should be made at a **wavelength of 450 nm** and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution**. Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	C 1	Co 2	P 8									
B	C 2	P 1	P 9									
C	C 3	P 2	P 10									
D	C 4	P 3	P 11									
E	C 5	P 4	P 12									
F	C 6	P 5	P 13									
G	C 7	P 6	P 14									
H	Co 1	P 7	P 15									

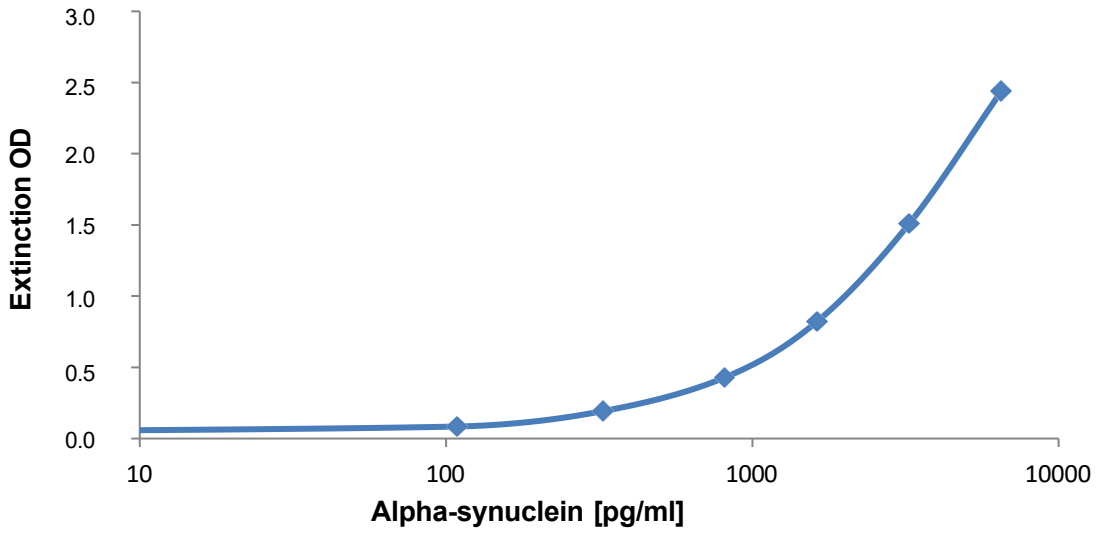
The pipetting protocol for microtiter strips 1 to 3 is an example for the **quantitative analysis** of 15 samples (P 1 to P 15).

The calibrators (C 1 to C 7), the controls (Co 1, Co 2), and the samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample.

The controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

Quantitative: The standard curve from which the alpha-synuclein concentrations in the CSF samples can be taken is calculated by “5PL” plotting (alternatively “4PL”, “Akima” or “cubic spline” can be used) of the extinction measured for the 7 calibrators against the corresponding units (linear/log). For correct logarithmic representation it might be necessary to set the concentration of calibrator 1 from 0 to e.g. 0.1 pg/ml. The following plot is an example of a typical calibrtion curve. Please do not use this curve for the determination of concentrations in samples.



If the extinction for a sample lies above the value of calibrator 7, it is recommended to retest the sample at an initial dilution of 1:4 in calibrator 1 before following the test instruction.

Note: Only use polypropylene (PP) tubes for dilution. Pipette calibrator 1 into tubes before adding sample. The result of sample in pg/ml read from the calibration curve for this sample must be multiplied by a factor of 4.

For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another, EUROIMMUN recommends retesting the samples.

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For the clinical evaluation the results must be confirmed with a CE-certified test.

Test characteristics

Calibration: The concentrations of calibrators and the acceptance ranges of controls are lot-dependent and given on the quality control certificate enclosed with this test instruction. For every group of tests performed, the values of the concentrations must lie within the limits stated for the relevant test kit lot. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated.

Antibodies: The reagent wells are coated with monoclonal anti-alpha-synuclein antibodies.

Detection limit:

Lower detection limit/Limit of Blank (LoB):	10 pg/ml
Lower detection/Limit of Detection (LoD):	24 pg/ml
Lower limit of quantitation/Limit of Quantitation (LoQ):	41 pg/ml

LoB, LoD and LoQ were defined according to the requirements defined in guideline EP17-A of the CLSI (Clinical and Laboratory Standards Institute, https://clsi.org/).