研究用試薬

Plasma Beta-Amyloid (1-42) ELISA
Instructions for use

ORDER NO.	ANTIGEN	SUBSTRATE	FORMAT
EQ 6521-9601	Beta-amyloid (1-42)	Ab-coated microplate wells	96 x 01 (96)

Principles of the test: In the first analysis step, the calibrators and diluted samples are diluted with biotinylated monoclonal anti-beta-amyloid (1-x) antibody and added to microplate wells coated with monoclonal anti-beta-amyloid (x-42) antibodies. In this process beta-amyloid (1-42) is bound in a complex. In a second incubation, streptavidin peroxidase conjugate binds the biotin. A following incubation with substrate and chromogen promotes a colour reaction. The colour intensity is proportional to the beta-amyloid (1-42) 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, plasma beta-amyloid (1-42), lyophilised	light red to dark red	1 x 600 µl	CAL 1
3. Calibrator 2, plasma beta-amyloid (1-42), lyophilised		1 x 600 µl	CAL 2
4. Calibrator 3, plasma beta-amyloid (1-42), lyophilised		1 x 600 µl	CAL 3
5. Calibrator 4, plasma beta-amyloid (1-42), lyophilised		1 x 600 µl	CAL 4
6. Calibrator 5, plasma beta-amyloid (1-42), lyophilised		1 x 600 µl	CAL 5
7. Calibrator 6, plasma beta-amyloid (1-42), lyophilised		1 x 600 µl	CAL 6
8. Control 1, plasma beta-amyloid (1-42), lyophilised	green	1 x 600 µl	CONTROL 1
9. Control 2, plasma beta-amyloid (1-42), lyophilised	blue	1 x 600 µl	CONTROL 2
10. Biotin, biotin-labelled plasma beta-amyloid detection antibody, ready for use	green	1 x 2.5 ml	BIOTIN
11. Sample buffer, ready for use	colourless	1 x 30 ml	SAMPLE BUFFER
12. Enzyme conjugate, peroxidase-labelled streptavidin, 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.

Updates with respect to the previous 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. 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 600 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. If necessary, shortly centrifuge vials to get remaining liquid from the cap into the tube. The reconstituted calibrators and controls must be frozen at -20 °C immediately after use. The calibrators and controls can be frozen and thawed up to three times. Longer residence times at room temperature must be avoided at all costs.
- **Biotin:** Lot-specific, ready for use.Mix thoroughly before use.
- **Enzyme conjugate:** Lot-specific, ready for use.Mix thoroughly before use.
- **Sample buffer:** Ready for use. 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: EDTA plasma.
NOTE: Use of EDTA plasma is mandatory as other types of plasma and serum will lead to incorrect results.

Notes on sample handling: After collection of blood in K_2EDTA tubes, blood samples may be stored for up to 3 hours at room temperature or alternatively up to 24 hours at +2 °C to +8 °C. After centrifugation (10 min and 1000xg at RT or +2 °C to +8 °C), aliquot plasma at 250-1000 µl in storage tubes made of polypropylene (PP) or protein low bind material. Prior to measurement, plasma samples may be stored at +2 °C to +8 °C for up to 24 hours. For longer time periods, samples must be stored at -20 °C or, alternatively, at -80 °C. Frozen samples may be thawed shortly at RT and must then be kept on ice. After measurement, samples should be frozen again (short-term: -20 °C; long-term: -80 °C).

Since preanalytical factors may have a severe impact on the measured concentration, it is recommended not to measure beta-amyloid (1-42) alone, but always in combination with beta-amyloid (1-40) and to calculate the beta-amyloid ratio.

研究用試薬

Samples should preferably not be subjected to more than one, at most two, freeze/thaw cycles.

Always use adequate personal protective equipment.

Performance: The **samples** for analysis are diluted 1:4 in sample buffer.
Example: Add 50 µl of EDTA plasma to 150 µl sample buffer and mix thoroughly (vortex).
NOTE: After reconstitution calibrators and controls are ready for use, do not dilute them further!

Incubation

Sample incubation: (1st step)

Pipette **20 µl** of biotin solution and **80 µl** of calibrators, controls and **1:4** diluted samples into each of the reagent wells. Slightly shake the microplate to ensure a homogeneous distribution of the solution.
Incubate for **180 minutes** 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: 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

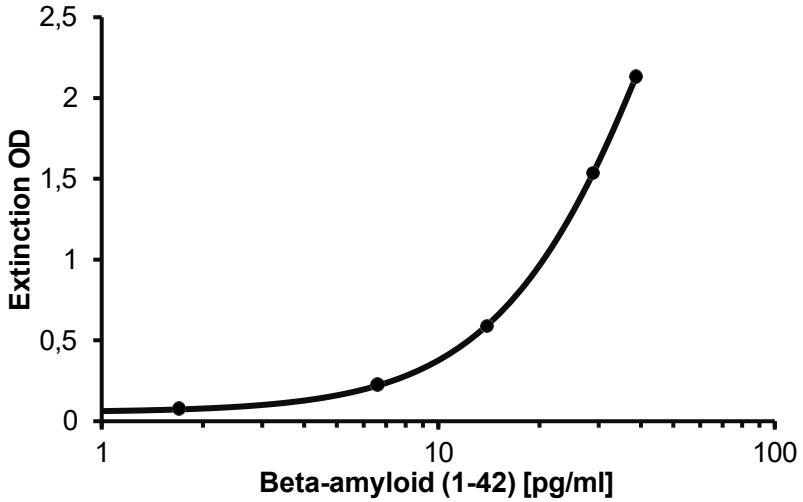
	1	2	3	4	5	6	7	8	9	10	11	12
A	**C 1**	**P 1**	**P 9**									
B	**C 2**	**P 2**	**P 10**									
C	**C 3**	**P 3**	**P 11**									
D	**C 4**	**P 4**	**P 12**									
E	**C 5**	**P 5**	**P 13**									
F	**C 6**	**P 6**	**P 14**									
G	**Co 1**	**P 7**	**P 15**									
H	**Co 2**	**P 8**	**P 16**									

The pipetting protocol for microplate strips 1 to 3 is an example for the **quantitative analysis** of 16 samples (P 1 to P 16).

The calibrators (C 1 to C 6), 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 must be assayed with each test run.

Calculation of results

Quantitative: The standard curve from which the beta-amyloid (1-42) concentrations in the EDTA plasma samples can be taken is calculated by “5PL” plotting (alternatively “4PL”, “Akima” or “cubic spline” can be used) of the extinction readings measured for the 6 calibrators against the corresponding units (linear/log). The samples have to be multiplied by the initial dilution factor 4.
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 calibration curve. Please do not use this curve for the determination of concentrations in samples.



If the extinction for a sample lies above the extinction of calibrator 6, it is recommended to retest the sample at an initial dilution of 1:8 in sample buffer before following the test instruction.

Note: Only use polypropylene (PP) or protein low bind tubes for dilution. Pipette sample buffer 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 8.

研究用試薬

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.

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-beta-amyloid (x-42) antibodies.

Measurement range: 9.1 pg/ml – 160* pg/ml
Limit of Blank (LoB): 2.4 pg/ml
Limit of Detection (LoD): 6.6 pg/ml
Limit of Quantitation (LoQ): 9.1 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/).

*The concentration of the highest calibrator and thus the upper limit of the measurement range are lot-specific.