INNO-BIA AlzBio3

RUO

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"Note changes Highlighted"

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INNOGENETICS®
BIOTECHNOLOGY FOR HEALTHCARE

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コスモ・バイオ株式会社

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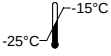
Symbols used

	Manufactured by
RUO	For research use only Not for use in diagnostic procedures
LOT	Lot number
REF	Catalogue number
	Use by
	Consult instructions for use
	Temperature limitation
COATED BEADS 100x	Coated beads 100x
CONJ 1 100x	Conjugate 1 100x
DETECT CONJ 100x	Detection Conjugate 100x
DIL	Diluent
READ SOLN	Reading Solution
WASH SOLN 25x	Wash Solution 25x
AlzBio3 Standard & Control	Packed in separate box with REF 80585 because of separate storage conditions
	Temperature limitation
STAND 1	Standard 1
STAND 2	Standard 2
STAND 3	Standard 3
STAND 4	Standard 4

STAND 5	Standard 5
STAND 6	Standard 6
CONTROL A	Control A
CONTROL B	Control B

English

Intended use

The INNO-BIA AlzBio3 is a quantitative assay for the simultaneous quantification of phosphorylated tau (P-tau$_{(181P)}$), tau, and ß-amyloid$_{(1-42)}$ (Aß$_{(1-42)}$) in human CSF.

For research use only. Not for use in diagnostic procedures.

Test principle

The INNO-BIA AlzBio3 is a fluorimetric immunoassay in which the different parameters are captured selectively on beads of a specific region number by a first monoclonal antibody (AT270 for P-tau$_{(181P)}$, AT120 for tau, 4D7A3 for Aß$_{(1-42)}$), coupled covalently.
The INNO-BIA AlzBio3 is tested using xMap® technology.
(For more information, see www.luminexcorp.com).

A mix of the microspheres is added in a specific volume to the filter plates. Cerebrospinal fluid (CSF) samples or standards are added on the filter plate, together with a mix of biotinylated antibodies (25-µl conjugate 1 working solution; 75-µl sample or standard).

Each biotinylated detector antibody detects one or several parameters (e.g. 3D6 for Aß$_{(1-42)}$, HT7 for detection of P-tau$_{(181P)}$ and tau).
The antigen-antibody complex is then detected by a phycoerythrin-labeled streptavidin conjugate. After a wash step, the solution is immediately measured in a Luminex® 100™ IS Total System, Luminex® 200™ IS Total System or Bio-Plex® 200 System.

The fluorescence intensity on a specific bead is related to the concentration of the parameter (= antigen) for which it was designed. The Luminex® or Bio-Plex® instrument analyzes microspheres in a flow stream. Based on its fluorescent signature, each microsphere is accurately classified to its own unique region.

In addition, each bead is scanned individually for the presence of a reporter fluorescence that quantifies the analyte at the surface of the bead.The excitation systems involve two solid state lasers. A 532-nm reporter laser excites fluorescent molecules bound to biological reactants at the microsphere surface (e.g. phycoerythrin molecules derived from the streptavidin conjugate), and a 635-nm classification laser excites fluorochromes embedded within the microsphere. These fluorescent signals are converted into intensity units by a digital signal processor.

Reagents

Description, preparation for use, and recommended storage conditions

- If kept at 2 - 8°C and stored in the original vials, all reagents, opened or unopened, are stable until the expiry date.
  Do not use the reagents beyond the expiry date.
  NOTE:
  - The AlzBio3 Standards and Controls are packed separately and must be stored at -20°C or lower upon arrival.
- All reagents, except the AlzBio3 Standards and Controls, should be brought to room temperature (18 - 30°C) approximately 30 minutes before use and should be returned to the refrigerator immediately after use.
- The AlzBio3 Standards and Controls should be thawed on the bench approximately 15 minutes before the start of the procedure and should be returned to the freezer (-20°C or lower) immediately after use.

Reagents supplied:

Component	Quantity	Ref	Description
Coated Beads 100x	0.13 ml	57823	xMap® microspheres of different regions (regions 2 for tau, 56 for Aß$_{(1-42)}$, and 69 for P-tau$_{(181P)}$), each coated with a monoclonal antibody specific to one of the parameters (resp. AT120 for tau, 4D7A3 for Aß$_{(1-42)}$, AT270 for P-tau$_{(181P)}$). Vortex, sonicate for 3 minutes, and vortex again before use. The coated beads must be diluted 100x with Diluent before being brought to the plate.
Conjugate 1 100x	0.045 ml	57824	A 100x-concentrated mixture of different biotinylated monoclonal antibodies, selected to detect specifically one of the parameters included in the kit (HT7 for tau and P-tau$_{(181P)}$; 3D6 for detection of Aß$_{(1-42)}$). Dilute 100x with Diluent before use. The conjugate 1 working solution must be prepared freshly for each test.

Detection Conjugate 100x	0.13 ml	57825	SV-PE (phycoerythrin-labeled streptavidin). Dilute 100x with Diluent before use (Remarks and precautions). Detection conjugate working solution must be prepared freshly for each test.
Diluent	45 ml	57826	Phosphate buffer with stabilizing proteins and 0.05% Proclin 300 as preservative, used to dilute conjugate 1, detection conjugate, and coated beads.
Reading Solution	15 ml	57827	Phosphate buffer containing 0.05% Proclin 300 as preservative.
Wash Solution 25x	45 ml	57882	Phosphate buffer containing 0.15% Proclin 300 as preservative, to be diluted 25x with distilled or deionized water before use. Prepare at least 20 ml diluted wash solution for each test well strip. Salt crystals may be formed in the concentrated wash solution after storage at 2 - 8°C. These crystals must be completely redissolved. The diluted wash solution is stable for 4 weeks if stored at 2 - 8°C.
Standard 1	0.6 ml	57828	Ready-to-use mixture of the standard for each parameter (tau, P-tau$_{(181P)}$, Aß$_{(1-42)}$).
Standard 2	0.6 ml	57829	Ready-to-use mixture of the standard for each parameter (tau, P-tau$_{(181P)}$, Aß$_{(1-42)}$).
Standard 3	0.6 ml	57830	Ready-to-use mixture of the standard for each parameter (tau, P-tau$_{(181P)}$, Aß$_{(1-42)}$).
Standard 4	0.6 ml	57831	Ready-to-use mixture of the standard for each parameter (tau, P-tau$_{(181P)}$, Aß$_{(1-42)}$).
Standard 5	0.6 ml	57832	Ready-to-use mixture of the standard for each parameter (tau, P-tau$_{(181P)}$, Aß$_{(1-42)}$).
Standard 6	0.6 ml	57874	Ready-to-use mixture of the standard for each parameter (tau, P-tau$_{(181P)}$, Aß$_{(1-42)}$).
Control A	0.6 ml	57833	Ready-to-use control sample
Control B	0.6 ml	57835	Ready-to-use control sample
Multiscreen 96-well filter plate	1	25343	Plate needed for the test performance
Adhesive plate Sealers	4	-	To cover unused wells
AlzBio3 Standard & Control concentrations	-	-	Target concentrations

Preparation for use

Preparation of diluted beadmix:

	8 wells	*16 wells*	*32 wells*	*64 wells*	*96 wells*
100 x coated beads in µl	10	20	40	80	120
DIL in ml	1	2	4	8	12

Preparation of conjugate 1 working solution and detection conjugate working solution:

	8 wells	*16 wells*	*32 wells*	*64 wells*	*96 wells*
CONJ 1 in µl	3	5	10	20	30
DIL in ml	0.3	0.5	1.0	2	3

	8 wells	*16 wells*	*32 wells*	*64 wells*	*96 wells*
DETECT CONJ in µl	10	20	40	80	120
DIL in ml	1	2	4	8	12

Preparation of diluted wash solution:

	8 wells	*16 wells*	*32 wells*	*64 wells*	*96 wells*
WASH SOLN 25x in ml	4	8	15	30	45
H_2O in ml	96	192	360	720	1080

Materials required but not provided

- Distilled or deionized water.
- Calibrated precision pipettes with disposable tip to deliver volumes in the range of 2 µl to 1000 µl. A calibrated multichannel pipette to deliver 25 µl, 75 µl, and 100 µl is recommended for addition of samples, conjugate 1 working solution, detection conjugate working solution, and Reading Solution.
- Vortex mixer or equivalent.
- Sonicator bath to sonicate the coated beads before use.
- Vacuum manifold (e.g. Millipore) to wash the plate.
- Disposable vials for preparation of working solutions.
- Luminex® 100™ IS Total System, Luminex® 200™ IS Total System or Bio-Plex® 200 System.
- Orbital plate shaker.

Safety and environment

- **Please refer to the Material Safety Data Sheet (MSDS) and product labelling for information on potentially hazardous components. The most recent MSDS version is available on the website www.innogenetics.com.**


R43, S23-24-37-60

Irritant! (Xi) May cause sensitization by skin contact.
Do noth breathe vapour/spray. Avoid contact with skin.
Wear suitable gloves. This material and its container must be disposed of as hazardous waste.

Contains 0.0018-0.0057% Mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one:
CONJ 1 100x, DIL, READ SOLN, WASH SOLN 25x.

- DETECT CONJ 100x contains sodium azide as preservative. To prevent the formation of very toxic gas, avoid contact of sodium azide with acids. To prevent the formation of explosive lead or copper azide in plumbing, thoroughly flush drains with water after disposal of solutions containing sodium azide."
- No test method can offer complete insurance that human CSF products will not transmit infectious agents. Therefore, all CSF samples and biological materials should be considered as being potentially infectious and should be handled as such. Only adequately trained personnel should be permitted to perform the test procedure. All CSF samples and biological materials should be disposed of in accordance with established safety procedures.
  - Autoclave for at least 15 minutes at 121°C.
  - Incinerate disposable material.
  - Mix liquid waste with sodium hypochlorite so that the final concentration is ± 1% sodium hypochlorite.
    Allow to stand overnight before disposal.
    CAUTION: Neutralize liquid waste that contains acid before adding sodium hypochlorite.
- Use of personal protective equipment is necessary: gloves and safety spectacles when manipulating dangerous or infectious agents.
- Waste should be handled according to the institution's waste disposal guidelines. All federal, state, and local environmental regulations should also be observed.

REMARK: Special precautions for transmissible spongiform encephalopathy (TSE)/prion-contaminated materials

- **Inactivation of samples**
  Clinical samples, e.g. CSF, should be autoclaved or immersed in a solution of sodium hypochlorite resulting in 20,000 ppm free chlorine for 1 hour before disposal by incineration.
- **Waste disposal**
  All material classified as clinical waste should be disposed of by incineration at an authorized incineration site.
  For the safe handling of clinical waste, use secure leak-proof containers, e.g. double bagging, where appropriate.
  Avoid external contamination of the container.
- Reference:
  - Advisory Committee on Dangerous Pathogens (UK) - Spongiform Encephalopathy Advisory Committee. Transmissible spongiform encephalopathy agents: Safe Working and the prevention of infection. Department of Health, UK 1998. PL CO (98/7).
  - World Health Organization (WHO): WHO Infection Control Guidelines for Transmissible Spongiform Encephalopathies. Report of a WHO Consultation, Geneva, Switzerland, 23-26 March 1999. WHO/CDS CSR/APH/2000.3.

Specimens

- Human cerebrospinal fluid stored at -20°C (-80°C preferably), free of cells, is collected in polypropylene tubes. Non-hemolytic.
- The assay is not designed for blood (serum, plasma) screening or cell culture supernatants.
- It is recommended to aliquot CSF samples to avoid probable effects of repeated freeze/thaw cycles on assay parameters. Repeated freeze/thaw cycles can result in incorrect concentration values.
- Insoluble material should be removed from all samples by centrifugation before testing, e.g. centrifuge at 4000 g for 10 minutes.

Remarks and precautions

- Do not mix components from kits of different lot numbers, except for the AlzBio3 Standard & Control.
- To ensure stability, it is necessary to protect the microspheres from daylight by storage in aluminum-covered tubes or brown/dark vials.

- All vessels used to prepare diluted beadmix, conjugate 1 working solution, and detection conjugate working solution must be thoroughly clean to avoid contamination.
- Hold the plate by the sides to avoid contamination of the wells.
- Avoid microbial contamination of reagents.
- Ensure that samples and standard solutions are homogeneous before use.
- Use a new pipette tip for each specimen.
- Ensure that specimen is added to the well.
- To avoid contamination, do not touch the edge of the wells with the pipette tips when adding sample or conjugate 1 working solution.
- Do not expose detection conjugate working solution to strong light.
- Always place the plate in the dark during incubation by wrapping it in aluminum foil.

Directions for washing

- Center the filter plate on the vacuum manifold by putting well A1 on the top left corner.
- Check the waste volume. If the waste is full, remove the waste first.
- Use plate sealers to cover unused wells.
- Push the plate against the instrument and press the ON button.
- The liquid must be aspirated completely from all wells.
- After aspiration, press the OFF button and fill the wells with 225 µl of diluted wash solution.
- Remove the liquid from the wells by pressing the ON button again, and pushing the plate. Gently dry the underside of the plate on absorbent tissue after aspiration.
- Repeat the above wash steps twice more, do not allow any time to elapse between wash steps.
- After the last aspiration step, dry the underside of the plate with an absorbent tissue.

Incomplete washing will adversely affect the test outcome. Microbial contamination of wash solution can cause extensive problems.

Test procedure

Please read remarks and precautions before performing the test.
NOTE:

- Determine the size of the assay by considering the total number of wells required. For each test run, duplicate wells of the 6 standards, one blank (Diluent), 2 controls, and CSF samples should be foreseen.
- Room temperature (RT) is defined as 18 - 30°C.

DAY 1

1. Allow all reagents to reach RT and let the standards thaw on the bench.
2. Vortex the coated beads and sonicate for 3 minutes.
3. After vortexing again, dilute the coated beads 100x in Diluent and cover this solution with aluminum foil while preparing the other reagents.
4. Vortex the thawed AlzBio3 Standards and Controls.
5. Prepare conjugate 1 working solution by diluting Conjugate 1 100x with Diluent.
6. Once the diluted beadmix, standards, samples, and conjugate 1 working solution are ready, the filter plate must be prewashed with 225 µl diluted wash buffer.
7. Transfer 100 µl (= 3000 beads/parameter) diluted beadmix to the wells of the filter plate.
8. Aspirate the filter plate by using the vacuum manifold.
   Dry the underside of the plate with an absorbent tissue.
9. Add 25 µl conjugate 1 working solution to the wells of the filter plate. Then add 75 µl of standards, controls, samples, and blanks to the appropriate wells. Cover the plate with aluminum foil.
10. Incubate overnight (14 to 18 hours) at RT on a orbital plate shaker.

DAY 2

11. Prepare detection conjugate working solution by diluting Detection Conjugate 100x with Diluent.
    Cover the solution with aluminum foil.
12. Aspirate the wells and wash each well 3 times with 225 µl diluted wash buffer.
    Using a multichannel pipette, add 100 µl detection conjugate working solution to each well. Cover the plate with aluminum foil.
13. Incubate 1 hour at RT on an orbital plate shaker.
14. After incubation, aspirate the wells and wash each well 3 times with 225 µl diluted wash buffer.
15. Add 100 µl Reading Solution to each well, cover the plate with aluminum foil, and place the filter plate for at least 2 minutes on an orbital plate shaker before measurement on the Luminex® 100™ IS Total System, Luminex® 200™ IS Total System or Bio-Plex® 200 System.

Results

Qualification

- Before reading, be sure that the daily maintenance procedure has been performed on the Luminex® 100™ IS Total System, Luminex® 200™ IS Total System or the Bio-Plex® 200 System.
- Calibration and control of the Luminex® 100™ IS Total System, Luminex® 200™ IS Total System and Bio-Plex® 200 System lasers should be performed on a regular basis.
- For further calculation of the results, the median fluorescence intensity values should be used.
- 100 beads of each region are counted.
- Ensure that the needle height of the Luminex® 100™ IS, Luminex® 200™ IS Total System or the Bio-Plex® 200 System is adjusted to a filter plate.

Test results

Calculate the mean of the median fluorescence intensity signal for standards and unknown samples.
Repeat the test if individual values differ by more than 20%.
Construct the standard curve by plotting the median values for each of the parameters on the vertical (Y) axis versus the corresponding parameter concentrations on the horizontal (X) axis.
Draw the best fitting curve through these points.

NOTE:

- A sigmoidal curve fitting is recommended (sigmoidal dose-response curve or four-parameter logistic equation).

Using the mean signal value of each unknown CSF sample, determine the corresponding concentration of each parameter from the relevant standard curve.

The concentration of samples can only be determined and reported if the median fluorescence intensity signal is within the range of the values obtained for the delivered standard vials. Extrapolation of results from signal values that lie above the highest standard point or below the lowest point of the standard curve can lead to incorrect results.

Recommended instrument settings

Luminex® 100™ IS Total System & Luminex® 200™ IS Total System

- Gate settings: 7500 to 15000
- Sample volume: 50 µl

Bio-Plex® 200 System

- The Bio-Plex® 200 System must be calibrated with Low RP1 target value. (The RP1 target values are listed on the label of the CAL2 bottle supplied by Bio-Rad)
- Gate settings: 4335 to 10000
- Sample volume: 50 µl

Limitation of the procedure

The INNO-BIA AlzBio3 assay procedure was designed to quantify tau, P-tau$_{(181P)}$, and Aß$_{(1-42)}$ in human cerebrospinal fluid. Insufficient data are available to interpret tests performed on other body fluids or brain tissue samples. Therefore, testing of such specimens with this test protocol is not recommended.

Disclaimer

A license regarding Amyloid beta antibodies contained in this product under patents US 570349 and EP 0683234 has been obtained from Takeda Pharmaceutical Company Limited.

Furthermore, a license for the use of Abeta monoclonal antibodies contained in this product under patents US 5,593,846A, US 5,766,846A, US 5,837,672A, US 6,284,221B1, US 6,610,493B1, US 5,441,870A, US 5,721,130A, US 5,605,811A and US 6,114,133A has been obtained from Eli-Lilly and Company.

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- Bio-Plex® 200 System is a registered trademark of Bio-Rad Laboratories Inc.