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Symbols used

Manufacturer

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

Batch code

Catalogue number

Use By

Consult Instructions for Use

Temperature limitation

Coated beads 100x

Conjugate 1 100x

Conjugate Diluent

Detection Conjugate 100x

Diluent

Plasma Diluent

Reading Solution

Wash Solution 25x

Warning
**Intended use**

The INNO-BIA plasma Aβ forms is an assay for the simultaneous quantification of human β-amyloid (Aβ) forms in plasma. This allows the calculation of the ratio of the amounts of the different Aβ forms present in the sample, eg, Aβ_{1-42}/Aβ_{1-40}. The numbers 1-40 and 1-42 indicate the length of the sequence of the Aβ peptide.

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

**Test principle**

Quantification of the different Aβ forms and calculation of their ratio is obtained after performing the test, provided in the kit.

The INNO-BIA plasma Aβ forms is a fluorimetric bead-based immunoassay using xMAP technology*. The different Aβ forms are captured selectively by a mix of beads (xMAP microspheres) that have been selectively coated with three monoclonal antibodies (mAb):

- Region 4: Detection of Aβ_{42} forms;
- Region 5: detection of Aβ_{40} forms;
- and Region 2: coupled with a non-Aβ binding mAb. Immunoreactivity with bead region 2 indicates the presence of heterophilic antibodies in the sample, which could result in a false positive result or artificially high values.

The bead mix is added to the filterplate, followed by aspiration of the buffer. Conjugate 1 is then added to the wells of the filterplate, together with standards, controls or plasma samples.

Conjugate 1 contains a second mAb that provides the specific quantification of Aβ_{1-42} and Aβ_{1-40} forms (Aβ1).
Figure 1: Test Principle
The sequence of the Aβ peptide and the epitopes of the selected mAbs are presented in figure 2.

![Diagram of test principle]

**Figure 2: Aβ peptide sequence and epitopes of the selected mAbs**
Following an incubation step, the antigen-antibody complex is then detected by a phycoerythrin-labeled streptavidin conjugate.
The mix is then washed and immediately analyzed using the Luminex 100 IS Total System, Luminex 200 IS Total System instrument or Bio-Plex 200 System.
The instrument analyzes microspheres in a flow stream.
The fluorescence intensity of a specific bead is related to the concentration of the bound antigen. Based on its fluorescent signature, each microsphere is accurately classified to a specific region.
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 unopened in the original vials, all reagents are stable until the expiry date. After opening, all reagents are stable for 8 months if kept at 2-8°C. Do not use the reagents beyond the expiry date.

**NOTE:** The Plasma Aβ forms Standards and Controls are packed separately and must be transferred to storage at -20°C or lower upon arrival. If kept at -15°C to -25°C and stored unopened in the original vials, all standard and controls are stable until the expiry date. After opening, all reagents are stable for 8 months if kept at -15°C to -25°C. Do not use standards and controls beyond the expiry date.
- All reagents, except the Plasma Aß forms 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 plasma Aß forms 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.
- Before opening, short spin the vial of coated beads 100x, conjugate 1 100x, and detection conjugate 100x. Keep period of opening as short as possible. Vials should be closed properly after use.

Reagents supplied:

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Ref.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coated Beads 100x</td>
<td>1x 0.26 mL</td>
<td>59910</td>
<td>xMAP microspheres specifically coated with monoclonal antibody to the different Aß regions: region 4 for Aß42 and region 5 for Aß40. Region 2 is a non-Aß-binding mAb. Vortex, sonicate for 3 minutes, and vortex again before use. The coated beads must be diluted 100x with Diluent before being added to the plate.</td>
</tr>
<tr>
<td>Conjugate 1 100x</td>
<td>2x 0.045 mL</td>
<td>59416</td>
<td>Contains the biotinylated monoclonal antibody for selective detection of Aß1 isoforms. Dilute 100x with Conj.Dil.1 before use. The conjugate 1 working solution must be prepared freshly for each test.</td>
</tr>
<tr>
<td>Detection Conjugate</td>
<td>1x 0.26 mL</td>
<td>59420</td>
<td>SV-PE (phycoerythrin-labeled streptavidin). Dilute 100x with Diluent before use (see Remarks and precautions) Detection conjugate working solution must be prepared freshly for each test.</td>
</tr>
<tr>
<td>Diluent</td>
<td>1x 55 mL</td>
<td>59422</td>
<td>Phosphate buffer with stabilizing proteins and 0.05% ProClin 300 as preservative, used to dilute coated beads and detection conjugate.</td>
</tr>
<tr>
<td>Plasma Diluent</td>
<td>1x 55 mL</td>
<td>59421</td>
<td>Buffer with stabilizing proteins, detergentia, and 0.05% Proclin 300 as preservative, used to dilute the plasma samples.</td>
</tr>
<tr>
<td>Conjugate Diluent 1</td>
<td>1x 15 mL</td>
<td>59467</td>
<td>Buffer with stabilizing proteins, mouse serum, and 0.05% Proclin 300 as preservative, used to dilute Conjugate 1.</td>
</tr>
<tr>
<td>Reading Solution</td>
<td>1x 40 mL</td>
<td>59423</td>
<td>Phosphate buffer containing 0.05% Proclin 300 as preservative.</td>
</tr>
</tbody>
</table>
### Component

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Ref.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wash Solution 25x</td>
<td>1x 45 mL</td>
<td>59424</td>
<td>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.</td>
</tr>
<tr>
<td>Standard 1</td>
<td>1x 1.2 mL</td>
<td>59409</td>
<td>Ready-to-use standard for Aβ₁₋₄₂ and Aβ₁₋₄₀.</td>
</tr>
<tr>
<td>Standard 2</td>
<td>1x 1.2 mL</td>
<td>59410</td>
<td>Ready-to-use standard for Aβ₁₋₄₂ and Aβ₁₋₄₀.</td>
</tr>
<tr>
<td>Standard 3</td>
<td>1x 1.2 mL</td>
<td>59411</td>
<td>Ready-to-use standard for Aβ₁₋₄₂ and Aβ₁₋₄₀.</td>
</tr>
<tr>
<td>Standard 4</td>
<td>1x 1.2 mL</td>
<td>59412</td>
<td>Ready-to-use standard for Aβ₁₋₄₂ and Aβ₁₋₄₀.</td>
</tr>
<tr>
<td>Standard 5</td>
<td>1x 1.2 mL</td>
<td>59413</td>
<td>Ready-to-use standard for Aβ₁₋₄₂ and Aβ₁₋₄₀.</td>
</tr>
<tr>
<td>Standard 6</td>
<td>1x 1.2 mL</td>
<td>59414</td>
<td>Ready-to-use standard for Aβ₁₋₄₂ and Aβ₁₋₄₀.</td>
</tr>
<tr>
<td>Control A</td>
<td>1x 1.2 mL</td>
<td>59417</td>
<td>Ready-to-use control sample</td>
</tr>
<tr>
<td>Control B</td>
<td>1x 1.2 mL</td>
<td>59418</td>
<td>Ready-to-use control sample</td>
</tr>
<tr>
<td>Multiscreen 96-well filter plate</td>
<td>2</td>
<td>25343</td>
<td>Plate for the test performance</td>
</tr>
<tr>
<td>Adhesive plate</td>
<td>4</td>
<td>-</td>
<td>To cover unused wells</td>
</tr>
<tr>
<td>Sealers</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Plasma Aβ forms</td>
<td>-</td>
<td>-</td>
<td>Target concentrations</td>
</tr>
<tr>
<td>Standard &amp; Control concentrations</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

### Preparation for use

**Preparation of diluted bead mix:**

<table>
<thead>
<tr>
<th>8 wells</th>
<th>16 wells</th>
<th>32 wells</th>
<th>64 wells</th>
<th>96 wells</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 x Coated Beads (µL)</td>
<td>10</td>
<td>20</td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td>DIL (mL)</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

**Preparation of Conjugate 1 working solution:**

<table>
<thead>
<tr>
<th>8 wells</th>
<th>16 wells</th>
<th>32 wells</th>
<th>64 wells</th>
<th>96 wells</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONJ 1 (µL)</td>
<td>3</td>
<td>5</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>CONJ DIL 1 (mL)</td>
<td>0.3</td>
<td>0.5</td>
<td>1.0</td>
<td>2</td>
</tr>
</tbody>
</table>
Preparation of Detection Conjugate working solution:

<table>
<thead>
<tr>
<th></th>
<th>8 wells</th>
<th>16 wells</th>
<th>32 wells</th>
<th>64 wells</th>
<th>96 wells</th>
</tr>
</thead>
<tbody>
<tr>
<td>DETECT CONJ (µL)</td>
<td>10</td>
<td>20</td>
<td>40</td>
<td>80</td>
<td>120</td>
</tr>
<tr>
<td>DIL (mL)</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>8</td>
<td>12</td>
</tr>
</tbody>
</table>

Preparation of diluted Wash solution:

<table>
<thead>
<tr>
<th></th>
<th>8 wells</th>
<th>16 wells</th>
<th>32 wells</th>
<th>64 wells</th>
<th>96 wells</th>
</tr>
</thead>
<tbody>
<tr>
<td>WASH SOLN 25x (mL)</td>
<td>4</td>
<td>8</td>
<td>15</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>H₂O (mL)</td>
<td>96</td>
<td>192</td>
<td>360</td>
<td>720</td>
<td>1080</td>
</tr>
</tbody>
</table>

Materials required but not provided

- Distilled or deionized water.
- Calibrated precision pipettes with disposable tips 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, Luminex 200 IS Total System instrument or Bio-Plex 200 System.
- Orbital plate shaker.

Safety and environment

Please refer to the Safety Data Sheet (SDS) and product labelling for information on potentially hazardous components. The most recent SDS version is available on the website [www.fujirebio-europe.com](http://www.fujirebio-europe.com).

**Warning**

Contains mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H-isothiazol-3-one
H317
P261-P280-P302+P352-P333+P313-P362+P364

**Hazard statements**

H317 May cause an allergic skin reaction.

**Precautionary statements**

P261 Avoid breathing mist/vapours/spray.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P302+P352 IF ON SKIN: Wash with plenty of soap and water.
P333+P313 If skin irritation or rash occurs: Get medical advice/attention.
P362+P364 Take off contaminated clothing and wash it before reuse.

- Only adequately trained personnel should be permitted to perform the test procedure.
- DETECT CONJ 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.
- CONJ DIL 1 contains mouse serum and should be handled as potentially infectious.
- No test method can offer complete insurance that human samples will not transmit infectious agents. Therefore, all samples and biological materials should be considered as being potentially infectious and should be handled as such. All 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.

**Inactivation of samples**
Clinical samples, e.g., CSF, blood, plasma, or serum should be autoclaved or immersed in a solution of sodium hypochlorite that provides 20000 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:***

**Specimens**
- Human plasma samples, free of cells and not hemolysed, stored in polypropylene tubes at -20°C or below (preferably -80°C), free of cells.
- The test is designed for EDTA plasma samples. There is not sufficient experimental evidence that the assay can be used for other plasma types or serum samples.
- The assay is not designed for CSF, brain homogenates, cell culture supernatants or other tissue types.
- It is recommended to aliquot plasma 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 4000g for 10 minutes.

Remarks and precautions
- Do not mix components from kits of different lot numbers.
- To ensure stability, it is necessary to protect the microspheres (beads) from daylight by storage in aluminum-covered tubes or light-proof vials.
- All vessels used to prepare diluted Bead Mix, 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.
- Ensure that the plate is kept 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 container and empty if necessary.
- Use plate sealers to cover unused wells.
- Push the plate down on the vacuum manifold 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 diluted wash solution.
- Remove the liquid from the wells by pressing the ON button again, and pushing the plate. Gently wipe the underside of the plate on absorbant 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 calculating the total number of wells required. For each test run, duplicate wells are necessary for the 6 standards, the blank (Plasma Diluent), the two controls, and the test plasma.
- Room temperature (RT) is defined as 18-30°C.
- Always add a blank (Plasma Diluent) to the filter plate in well A1.

**DAY 1**

1. Allow all reagents to reach RT and let the standards, controls, and plasma samples thaw on the bench. Limit the time period on the bench to less than one hour.
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 ‘Plasma Aβ forms Standards and Controls’.
5. Prepare Conjugate 1 working solution by diluting [Conjugate 1 100x] with [Conjugate Diluent 1].
6. After vortexing, dilute the plasma samples 3x in Plasma Diluent. Vortex the diluted plasma samples.
7. Once the diluted bead mix, standards, controls, diluted plasma samples, and Conjugate 1 working solution are ready, the filter plate must be pre-washed with 225 µL diluted wash buffer.
8. Transfer 100 µL ( = 3000 beads/parameter) diluted Bead Mix to the wells of the filter plate.
9. Aspirate the filter plate using the vacuum manifold. Gently dry the underside of the plate with an absorbent tissue.
10. Add 25 µL Conjugate 1 working solution to the corresponding wells of the filter plate. Then add 75 µL of standards, controls, diluted plasma samples, and blanks to the appropriate wells. Cover the plate with aluminum foil.
11. Incubate overnight (14 to 18 hours) at 2-8°C on an orbital plate shaker.

**DAY 2**

12. Prepare detection conjugate working solution by diluting [Detection Conjugate 100x] with [Diluent]. Cover the solution with aluminum foil.
13. Aspirate the wells and wash each well 3 times with 225 µL diluted wash buffer.
14. Add 100 µL Detection Conjugate working solution to each well. Cover the plate with aluminum foil and incubate 1 hour at RT on an orbital plate shaker.
15. After incubation, aspirate the wells and wash each well 3 times with 225 µL diluted wash buffer.
16. 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 Bio-Plex 200 System.
- Calibration and control of the instrument lasers should be performed on a regular basis.
- For further calculation of the results, the **median fluorescence intensity** values should be used.
- Minimum required counted beads per region ≥ 50.
- Ensure that the needle height of the Luminex 100 IS Total System, Luminex 200 IS Total System or 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 analysis for a sample if individual values (replicate testing) differ by more than 20%.

Construct the standard curve by plotting the median fluorescence values for each of the standard Aβ forms on the vertical (Y) axis against its corresponding concentration 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 plasma sample, determine the corresponding concentration of each Aβ form 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 and Luminex 200 IS Total System**
- Gate settings: 7500 to 15000
- Sample volume: 50 µL
- Count per region: 100 beads

**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 setting: 4335 to 10000
- Sample volume: 50 µL

**Limitation of the procedure**

The INNO-BIA plasma Aβ forms assay procedure was designed to quantify Aβ1-42 and Aβ1-40 in human plasma samples. Insufficient data are available to interpret tests performed on other body fluids, cell culture medium, or tissue samples.

**Disclaimer**

A license regarding amyloid beta antibodies contained in this product under patents US 5750349 has been obtained from Takeda Pharmaceutical Company Limited.

Furthermore, a license for the use of amyloid beta monoclonal antibodies contained in this product under patents US 6114133, US 7811769 and EP 0792458 has been obtained from Eli-Lilly and Company.
Trademarks

- INNO-BIA is worldwide a trademark™ such as in USA.
- xMAP® is a registered trademark of Luminex Corporation.
- Luminex® 100™ IS Total System and Luminex® 200™ IS Total System are registered trademarks of Luminex Corporation.
- Bio-Plex® 200 System is a registered trademark of Bio-Rad Laboratories Inc.
- ProClin® 300 is a registered trademark of Rohm and Haas Company.