Amyloid-β (1-x) ELISA

Enzyme immunoassay for the quantitative determination of human amyloid-β (1-x) in CSF, serum, EDTA plasma, cell culture supernatant and extracts from brain tissue

REF JP27729

Σ 12 x 8

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.
Human Amyloid β (1-16) Assay Kit - IBL

INTRODUCTION
Alzheimer’s disease (AD) was first reported by A. Alzheimer, a German neuropathologist in 1907 and is considered as a major factor of dementia. Senile plaque observed in the Alzheimer brain consists of Amyloid β protein (Aβ). It is reported that Aβ protein consists of 40-42 (43) amino acids, and that (1-16) variants which is cleaved from Amyloid Precursor Protein (APP; which exists in three main isoforms, APP695, APP751, and APP770) by β-secretase and subsequent γ-secretase (ref. 1). Reports have shown many variants of Aβ exist and are clarified into the culture supernatant from the APP cDNA transfected mouse neuroblastoma cell. (ref. 2).

In addition, you can use Code No.27711 Human Amyloid β (1-16) Assay Kit or Code No.27718 Human Amyloid β (1-40) (FL) Assay Kit, to measure Aβ (1-40), which held C-terminal side completely.

As stated above, it is useful for research of AD to measure each of Aβ (1-40), Aβ (1-42) and Aβ (N3pE-42), separately.

IBL Amyloid β Product Lines:

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Name</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>27711</td>
<td>Human Amyloid β (1-142) Assay Kit - IBL</td>
<td>96 Well</td>
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<tr>
<td>27712</td>
<td>Human Amyloid β (1-42) (N) Assay Kit - IBL</td>
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<td>27713</td>
<td>Human Amyloid β (1-140) Assay Kit - IBL</td>
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<td>27714</td>
<td>Human Amyloid β (1-40) (N) Assay Kit - IBL</td>
<td>96 Well</td>
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<td>27716</td>
<td>Human Amyloid β (N3pE) Assay Kit - IBL</td>
<td>96 Well</td>
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<td>27718</td>
<td>Human Amyloid β (1-40) (FL) Assay Kit - IBL</td>
<td>96 Well</td>
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<tr>
<td>27279</td>
<td>Human Amyloid β (1-16) Assay Kit - IBL</td>
<td>96 Well</td>
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</table>

PRINCIPLE
This kit is a solid phase sandwich ELSIA using 2 kinds of high specific antibodies. Tetra Methyl Benzidine (TMB) is used as coloring agent (Chromogen). The strength of coloring is in proportion to the quantities of human Aβ (1-40).

MEASUREMENT RANGE
7.81 ~ 500 pg/mL  
(1.8 ~ 115.5 pmol/L, as molecular weight of Aβ (1-40) is 4331)

INTENDED USE
■ The IBL’s Human Amyloid β (1-16) Assay Kit is a complete kit for the quantitative determination of human Aβ (1-16) in EDTA-plasma, cerebrospinal fluids, serum, cell culture media or the extract from brain tissue.

Aβ (1-16) in serum is very unstable. Since reduction in measured value is accepted according to a preservation situation.

If FCS etc. is contained in samples of culture supernatant, Aβ (1-16)-like in FCS may be measured. We recommend you to take the negative control.

Both recombinant and native forms of human Aβ (1-16) can be detected with the kit.

KIT COMPONENT

1. Precocated plate  
   - Anti-human Aβ (N3pE) Mouse IgG MAb Affinity Purified  
   - EDTA-plasma, cerebrospinal fluids, serum, cell culture media, or brain extraction.

2. Labeled antibody Conc.  
   - HRP conjugated Anti-human Aβ (11-28) Mouse IgG MAb Affinity Purified (X30)  
   - 0.4mL x 1

3. Standard  
   - Human Aβ (1-40)  
   - 1.0mL x 2

4. EIA buffer  
   - 1% BSA, 0.05% Tween 20 in PBS  
   - 30mL x 1

5. Solution for Labeled antibody: 1% BSA, 0.05% Tween 20 in PBS  
   - 12mL x 1

6. Chromogen  
   - TMB solution  
   - 15mL x 1

7. Stop solution  
   - 1N H2SO4  
   - 12mL x 1

8. Wash buffer Conc.  
   - 0.05% Tween 20 in phosphate buffer (X40)  
   - 50mL x 1

OPERATION MANUAL

1. Materials needed but not supplied
   - Plate reader (450nm)  
   - Micropipette and tip  
   - Graduated cylinder and beaker  
   - Distilled water  
   - Refrigerator (as 4℃)  
   - Graph paper (log/log)  
   - Paper towel  
   - Tube for dilution of Standard  
   - Washing bottle for precoated plate  
   - Disposable test tube for "2, Labeled antibody Conc." and "6, Chromogen"

2. Preparation
   1) Preparation of wash buffer  
      - "8, Wash buffer Conc." is a concentrated (X40) buffer. The temperature of "8, Wash buffer Conc." shall be adjusted to room temperature and then, mix it gently and completely before use. Dilute 50mL of "8, Wash buffer Conc." with 1,950mL of distilled water and mix it. This is the wash buffer for use. Prepared wash buffer shall be stored in refrigerator and used within 2 weeks after dilution.
   - Preparation of Labeled antibody  
      - "2, Labeled antibody Conc." is a concentrated (X30). Dilute 2, Labeled antibody Conc." with “5, Solution for Labeled antibody” in 30 times according to required quantity into a disposable test tube. Use this resulting solution as Labeled antibody.

Example:
In case you use one silt (8 well), the required quantity of Labeled antibody is 800 µL. (Dilute 30 µL of "2, Labeled antibody Conc." with 870 µL of "5, Solution for Labeled antibody" and mix it. And use the resulting solution by 100 L in each well.)

This operation should be done just before the application of Labeled antibody.

The remaining "2, Labeled antibody Conc." should be stored at 4℃ in firmly sealed vial.

3) Preparation of Standard
   - Put just 1.9 mL of distilled water into the vial of "3, Standard" and mix it gently and completely. The solution is 1,000 pg/mL Human Aβ (1-40) standard.

4) Dilution of Standard
   - Prepare 8 tubes for dilution of "3, Standard". Put 230 µL each of "4, EIA buffer" into the tube.
   - Specify the following concentration of each tube.

<table>
<thead>
<tr>
<th>Tube</th>
<th>Concentration</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>500 pg/mL</td>
<td>0.5 µL</td>
</tr>
<tr>
<td>2</td>
<td>250 pg/mL</td>
<td>0.5 µL</td>
</tr>
<tr>
<td>3</td>
<td>125 pg/mL</td>
<td>0.5 µL</td>
</tr>
<tr>
<td>4</td>
<td>62.5 pg/mL</td>
<td>0.5 µL</td>
</tr>
<tr>
<td>5</td>
<td>31.25 pg/mL</td>
<td>0.5 µL</td>
</tr>
<tr>
<td>6</td>
<td>15.63 pg/mL</td>
<td>0.5 µL</td>
</tr>
<tr>
<td>7</td>
<td>7.81 pg/mL</td>
<td>0.5 µL</td>
</tr>
<tr>
<td>8</td>
<td>0 pg/mL</td>
<td>0.5 µL</td>
</tr>
</tbody>
</table>

Put 230 µL of Standard solution into tube-1 and mix it gently. Then, put 230 µL of tube-1mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 500 pg/mL and 7.81 pg/mL. Tube-8 is the test sample blank as 0 pg/mL.

See following picture.

5) Dilution of test sample
   - Test sample may be diluted with "4, EIA buffer" if the need arises.
   - If the concentration of Aβ in samples may not be estimated in advance, the pre-assay with several different dilutions will be recommended to determine the proper dilution of samples.

3. Measurement procedure
   - All reagents shall be brought to room temperature approximately 30 minutes before use. Then mix it gently and completely before use. Confirm no change in quality of the reagents. Standard curve shall be prepared simultaneously with the measurement of test samples.

   **Reagents**
   - Test Sample: Standard  
   - 100 µL  
     - Diluted Standard (Tube 1~7)  
     - 100 µL  
     - EIA buffer (Tube-8)  
     - 100 µL

   **Incubation for overnight at 4℃ with plate lid**

   **Washing 9 times**

   **Chromogen**
   - 100 µL
   - 100 µL
   - 100 µL  
   - 100 µL
   - 100 µL
   - 100 µL
   - 100 µL
   - Read the plate at 450nm within 30 minutes after application of Stop solution.

   1) Determine well for reagent blank. Put 100 µL each of "4, EIA buffer" into the wells.
   - 2) Determine well for test sample blank, test sample and diluted standard. Then, put 100 µL each of test sample blank (tube-8), test sample and dilutions of standard (tube-1~7) into the appropriate wells.
   - 3) Incubate the precoated plate for overnight at 4℃ after covering it with plate lid.
   - 4) Wash each well of the precoated plate vigorously with wash buffer using washing bottle. Then, fill each well with wash buffer and place the precoated plate for 15~30 seconds. Remove wash buffer completely from the precoated plate by snapping. This procedure must be repeated more than 7 times, then, remove the remaining fluid from all wells completely by snapping the precoated plate onto paper towel.
   - In case of using plate washer, after 4 times washing with above washing bottle must be repeated 3 times.
   - 5) Pipette 100 µL of labeled antibody solution into the wells of test samples, diluted standard and test sample blank.
   - 6) Incubate the precoated plate for 1 hour at 4℃ after covering it with plate lid.
   - 7) Wash the precoated plate 9 times in the same manner above 4).
   - 8) "6, Chromogen" should be taken the required quantity into a disposable test
2. Added Recovery Assay

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Theoretical Value (pg/mL)</th>
<th>Measurement Value (pg/mL)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% FCS added RPMI-1640 (x4)</td>
<td>268.04</td>
<td>257.00</td>
<td>104.3</td>
</tr>
<tr>
<td>Human Serum (x4)</td>
<td>141.55</td>
<td>132.00</td>
<td>94.2</td>
</tr>
<tr>
<td>Human Plasma (EDTA) (x4)</td>
<td>80.08</td>
<td>92.05</td>
<td>114.8</td>
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<tr>
<td>Human Cerebrospinal fluids (x4)</td>
<td>58.40</td>
<td>60.80</td>
<td>102.0</td>
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<tr>
<td></td>
<td>42.57</td>
<td>45.18</td>
<td>106.3</td>
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<tr>
<td></td>
<td>99.51</td>
<td>118.21</td>
<td>119.2</td>
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</table>
| 3. Inter-Assay

<table>
<thead>
<tr>
<th>Measurement Value (pg/mL)</th>
<th>SD value</th>
<th>CV value (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>436.65</td>
<td>8.57</td>
<td>2.0</td>
<td>24</td>
</tr>
<tr>
<td>98.57</td>
<td>5.54</td>
<td>5.6</td>
<td>24</td>
</tr>
<tr>
<td>32.66</td>
<td>2.50</td>
<td>7.6</td>
<td>24</td>
</tr>
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5. Specificity

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human β (1-40)</td>
<td>100.0%</td>
</tr>
<tr>
<td>Human β (1-42)</td>
<td>100.0%</td>
</tr>
<tr>
<td>Human β (1-16)</td>
<td>≤0.1%</td>
</tr>
<tr>
<td>Human β (1-28)</td>
<td>100.0%</td>
</tr>
<tr>
<td>Human β (3-40)</td>
<td>≤0.1%</td>
</tr>
<tr>
<td>Human β (1-40)</td>
<td>≤0.1%</td>
</tr>
<tr>
<td>Human β (2-40)</td>
<td>≤0.1%</td>
</tr>
<tr>
<td>Human β (3-40)</td>
<td>≤0.1%</td>
</tr>
</tbody>
</table>

6. Sensitivity

3.55 pg/mL

The sensitivity for this kit was determined using the guidelines under the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols. (National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.

PRECAUTION FOR INTENDED USE AND/OR HANDLING

1. All reagents should be stored at 2~8°C. All reagents shall be brought to room temperature approximately 30 minutes before use.
2. “Standard” is lyophilized products. Be careful to open this vial.
3. “7, Stop solution” is a strong acid substance. Therefore, be careful not to contact your skin and clothes with “7, Stop solution” and pay attention to the disposal of “7, Stop solution”.
4. “1, Precalculated plate” and “3, Standard” contain sodium azide. Therefore, dispose these materials after diluting them with large quantity of water to avoid the production of explosive metallic azide.
5. The precipitation may grow in “2, Labeled antibody Conc.”, however, there is no problem in the performance.
6. Wash hands after handling reagents.
7. Do not mix the reagents with the reagents from different lot or different kit.
8. Do not use the reagents expired.
9. This kit is for research purpose only. Do not use for clinical diagnosis.

STORAGE AND THE TERM OF VALIDITY

Storage Condition : 2 ~ 8°C
The term of validity : 12 months
(The expiry date is specified in outer box.)

REFERENCE


Version 051001 Established

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Symbols / Symbole / Symbôles / Símbolos / Σύμβολα

**LIABILITY:** Complaints will be accepted in each mode – written or vocal. Preferred is that the complaint is accompanied with the test performance and results. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement. Regardless, in the event of any claim, the manufacturer’s liability is not to exceed the value of the test kit. Any damage caused to the kit during transportation is not subject to the liability of the manufacturer.

Symbols Version 3.5 / 2011-07-01