INTRODUCTIONS
Alzheimer’s disease (AD) was first reported by A. Alzheimer, a German neuropathologist in 1907 and is considered as a major factor of dementia. It is known that Amyloid β (Aβ; which is major constituent of senile plaque) 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). The production of soluble APPβ (sAPPβ) by β-secretase cleavage corresponds to Aβ production accordingly, so it is desired to measure sAPPβ in parallel with Aβ. In addition, it is reported that APP gene mutation exists in individuals who suffer early-onset familial Alzheimer’s disease. Swedish mutation, one of the APP gene mutations, is a double mutation at positions -1 to -2 from the β-secretase cleavage site (Lys757→Asn and Met717→Leu). And further, it is reported that Swedish mutation elevates Aβ40 and Aβ42 production (ref. 2), and that the mutation is utilized in establishment of transgenic mice (ref. 3). The measuring sAPPβ in Swedish type is useful for research of AD as well as in wild type. On the one hand, it is considered that in the metabolic pathway of APP, APP is first cleaved by α-secretase rather than β-secretase normally to produce soluble APPα (sAPPα) and subsequently P3 is cleaved from the remaining C-terminal fragment by γ-secretase. This kit can measure human soluble sAPPβ wild type (sAPPβ-w) in samples. IBL has many other kinds of Amyloidβ-related products for AD research. They are very specific assay systems for each target and they can be used according to the purpose of study.

PRINCIPLE
This kit is a solid phase sandwich ELISA using 2 kinds of high specific antibodies. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The strength of coloring is proportional to the quantities of Human sAPPβ-w.

MEASUREMENT RANGE
0.78 - 50 ng/mL

INTENDED USE
For research use only, not for use in diagnostic procedures.

This IBL's assay kit is capable for the quantitative determination human sAPPβ-w in EDTA plasma, cerebrospinal fluids and cell culture supernatant.

The recommended dilution for EDTA plasma is more than 4-fold. However, the value of normal person’s plasma is expected to be below the measurement range.

The recommended dilution for cerebrospinal fluids is more than 8-fold.

When the culture media samples contain serum like FCS, cross-reaction may be observed. So we recommend you to set the negative medium control.

KIT COMPONENT

1. Materials needed but not supplied
   - Plate reader (450nm)
   - Graph paper (log/log)
   - Disposable test tube for "2, Labeled antibody Conc." and "6, Chromogen"
   - Micropipette and tip
   - Deionized water
   - Stop solution*
   - Reagent blank
   - Assay buffer (100 μL)
   - Microtiter plate
   - Dilution of Standard

2. Preparation
   1) Preparation of wash buffer
      • "Wash buffer Conc." is a concentrated (40X) buffer. Adjust the temperature of "8, Washing buffer Conc." to room temperature and then, mix it gently and completely before use. Dilute 50 mL of "Wash buffer Conc." with 1,950 mL of deionized water and mix it. This is the wash buffer for use. This prepared wash buffer shall be stored in refrigerator and used within 2 weeks after dilution.
   2) Preparation of Labeled antibody
      "2, Labeled antibody Conc." is a concentrated (30X). Dilute "2, Labeled antibody Conc." with 5 ml of Diluent 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 strip (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 μL 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°C in firmly sealed vial.
   3) Preparation of Standard
      Put just 0.5 mL of deionized water into the vial of "3, Standard" and mix it gently and completely. This solution is 100 ng/mL Human sAPPβ-w.
   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.

   Tube 1:  50 ng/mL
   Tube 2:  25 ng/mL
   Tube 3:  12.5 ng/mL
   Tube 4:  6.25 ng/mL
   Tube 5:  3.13 ng/mL
   Tube 6:  1.56 ng/mL
   Tube 7:  0.78 ng/mL
   Tube 8:  0 ng/mL (Test Sample Blank)

   Put 230 μL of Standard solution into tube-1 and mix it gently. Then, put 230 μL of tube-1 mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 50 ng/mL and 0.78 ng/mL. Tube-8 is the test sample blank as 0 ng/mL.

   See following picture.

5) Dilution of test sample
   Test samples should be diluted with "4, EIA buffer" as necessary. If the concentration of human sAPPβ-w 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. Make sure of no change in quality of the reagents. Standard curve shall be prepared simultaneously with the measurement of test samples.

   Reagents
   Test Sample Standard Test Sample Blank Reagent Blank
   Test sample 100 μL Diluted standard (Tube 1~7) 100 μL EIA buffer (Tube-6) 100 μL EIA buffer 100 μL
   Incubation overnight at 4°C with plate lid
   Labeled Antibody 100 μL 100 μL 100 μL -
   Incubation for 30 minutes at 4°C with plate lid
   Chromogen 100 μL 100 μL 100 μL 100 μL
   Incubation for 30 minutes at room temperature (shielded)
   Stop solution 100 μL 100 μL 100 μL 100 μL
   Read the plate at 450nm against a Reagent Blank within 30 minutes after addition of Stop solution.

1) Determine wells for reagent blank. Put 100 μL each of "4, EIA buffer" into the wells.
2) Determine wells for test sample blank, test sample and diluted standard. Then, put 100 μL each of test sample blank (tube-8), test sample and dilutions (tube-1~7) into the appropriate wells.
3) Incubate the precoated plate overnight at 4°C after covering it with plate lid.
4) Wash the plate with the prepared wash buffer and remove all liquid.
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 30 minutes at 4°C after covering it with plate lid.
7) Wash the plate with the prepared wash buffer and remove all liquid.
8) Take the required quantity of "6, Chromogen" into a disposable test tube. Then, pipette 100 μL from the test tube into the wells. Please do not return the rest of the test tube to "6, Chromogen" bottle to avoid contamination.
9) Incubate the precoated plate for 30 minutes at room temperature in the dark.
10) Pipette 100 μL of "7, Stop Solution" into the wells. Mix the liquid by tapping the side of precoated plate. The liquid will turn yellow by addition of "7, Stop solution".
11) Remove any dirt or drop of water on the bottom of the precoated plate and confirm there is no bubble on the surface of the liquid. Then, run the plate reader and conduct measurement at 450 nm against a reagent blank.
   The measurement shall be done within 30 minutes after addition of "7, Stop solution".

   SPECIAL ATTENTION
1) Test samples should be measured soon after collection. For the storage of test samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before measurement.
2) Test samples should be diluted with "4, EIA buffer", if the need arises.
3) Duplicate measurement of test samples and standard is recommended.
4) Use the test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
5) Use only wash buffer contained in this kit for washing the precoated plate.
Insufficient washing may lead to the failure in measurement.
6) Remove the wash buffer completely by tapping the precoated plate on paper towel. Do not wipe wells with paper towel.
7) "6. Chromogen" should be stored in the dark due to its sensitivity against light.
8) "6. Chromogen" should be avoided contact with metals.
9) Measurement should be done within 30 minutes after addition of "7, Stop solution".

CALCULATION OF TEST RESULT
Subtract the absorbance of test sample blank from all data, including standards and unknown samples before plotting. Draw the best smooth curve through these points to construct the standard curve. Read the concentration for unknown samples from the standard curve.

Example of standard curve

![Graph showing standard curve]

The typical standard curve is shown above. This curve can not be used to derive test results. Please run a standard curve for each assay.

PERFORMANCE CHARACTERISTICS

1. Titer Assay (Samples with standard added are used.)

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Titer (X)</th>
<th>Measurement Value (ng/mL)</th>
<th>Theoretical Value (ng/mL)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 % FCS added RPMI-1640</td>
<td>2</td>
<td>15.27</td>
<td>25.00</td>
<td>61.1</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>11.78</td>
<td>12.50</td>
<td>94.2</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>6.82</td>
<td>6.25</td>
<td>109.1</td>
</tr>
<tr>
<td>Human Plasma (EDTA)</td>
<td>2</td>
<td>8.58</td>
<td>13.38</td>
<td>64.1</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5.20</td>
<td>6.67</td>
<td>78.7</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>3.10</td>
<td>3.41</td>
<td>90.9</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>2.10</td>
<td>2.11</td>
<td>97.6</td>
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<tr>
<td>Human Cerebrospinal fluids</td>
<td>2</td>
<td>12.07</td>
<td>12.50</td>
<td>96.6</td>
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<td></td>
<td>4</td>
<td>6.70</td>
<td>6.25</td>
<td>107.2</td>
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2. Added Recovery Assay

<table>
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<th>Specimen</th>
<th>Theoretical Value (ng/mL)</th>
<th>Measurement Value (ng/mL)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% FCS added RPMI-1640 (x2)</td>
<td>12.72</td>
<td>8.43</td>
<td>66.3</td>
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<tr>
<td></td>
<td>6.47</td>
<td>4.29</td>
<td>66.3</td>
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<td></td>
<td>3.34</td>
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<td>3.54</td>
<td>2.30</td>
<td>62.1</td>
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<tr>
<td></td>
<td>1.98</td>
<td>1.22</td>
<td>61.6</td>
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<tr>
<td></td>
<td>1.20</td>
<td>0.81</td>
<td>67.5</td>
</tr>
<tr>
<td>Human Plasma (EDTA) (x4)</td>
<td>12.79</td>
<td>9.91</td>
<td>77.5</td>
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<td></td>
<td>6.54</td>
<td>5.03</td>
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<td></td>
<td>3.42</td>
<td>2.59</td>
<td>75.7</td>
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3. Intra - Assay

<table>
<thead>
<tr>
<th>Measurement Value (ng/mL)</th>
<th>SD value</th>
<th>CV value (%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.43</td>
<td>0.46</td>
<td>2.8</td>
<td>24</td>
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<tr>
<td>6.96</td>
<td>0.29</td>
<td>4.2</td>
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<tr>
<td>2.45</td>
<td>0.09</td>
<td>3.7</td>
<td>24</td>
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4. Inter - Assay

<table>
<thead>
<tr>
<th>Measurement Value (ng/mL)</th>
<th>SD value</th>
<th>CV value (%)</th>
<th>n</th>
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</thead>
<tbody>
<tr>
<td>19.60</td>
<td>1.71</td>
<td>8.7</td>
<td>6</td>
</tr>
<tr>
<td>8.29</td>
<td>0.74</td>
<td>8.9</td>
<td>6</td>
</tr>
<tr>
<td>2.90</td>
<td>0.29</td>
<td>10.0</td>
<td>6</td>
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5. Specificity

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human sAPPβ-w</td>
<td>100 %</td>
</tr>
<tr>
<td>Human sAPPβ-sw</td>
<td>0.25 %</td>
</tr>
<tr>
<td>Human sAPPα</td>
<td>1.41 %</td>
</tr>
</tbody>
</table>

6. Sensitivity

0.05 ng/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.)