Human sAPPβ-sw (highly sensitive) 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. 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, APP711, 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 desirable to measure sAPPβ in parallel with Aβ. In addition, it is reported that APP gene mutation exists in early-onset familial Alzheimer’s disease patient. Swedish mutation, one of the APP gene mutations, is a double mutation at positions -1 to -2 from the addition, it is reported that APP gene mutation exists in early-onset familial Alzheimer’s disease patient. Swedish mutation, one of the APP gene mutations, is a double mutation at positions -1 to -2 from

sAPPβ cleavage corresponds to Aβ production. Therefore, it is desirable to measure sAPPβ in parallel with Aβ. In addition, it is reported that Swedish mutation exists in early-onset familial

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

MEASUREMENT RANGE 0.39 - 25 ng/mL

INTENDED USE
This IBL’s assay kit is capable for the quantitative determination human sAPPβ-sw in EDTA plasma of the Tg2576 transgenic mouse and cell culture supernatant.

KIT COMPONENT
1 Precoated plate
2 Labeled antibody Conc. : Anti Human sAPPβ-Swedish Type Rabbit IgG Affinity Purify 96Well x 1
3 Standard : Recombinant human sAPPβ-Swedish type protein 0.4 mL x 1
4 EIA buffer 30 mL x 1
5 Solution for Labeled antibody 125 mL x 1
6 Chromogen : TMB solution 15 mL x 1
7 Stop solution 12 mL x 1
8 Wash buffer Conc.* 50 mL x 1

OPERATION MANUAL
1. Materials needed but not supplied
   • Plate reader (450nm)
   • Micro pipette and tip
   • Graduated cylinder and beaker
   • Refrigerator (as 4°C)
   • Paper towel
   • Disposable test tube for Labeled antibody and Chromogen

2. Preparation
   1) Preparation of wash buffer
      “8, Wash buffer Conc.” is a concentrated (40X) buffer. Adjust the temperature of “8, Wash buffer Conc.” to room temperature and then, mix it gently and completely before use. Dilute 50 mL of “8, Wash buffer Conc.” with 1.5 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, 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 strip (8 well), the required quantity of Labeled antibody is 800 μL. (Dilute 30 μL of “2, Labeled antibody Conc.” with 670 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 50 ng/mL human sAPPβ-sw 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.
      Tube-1 25 ng/mL
      Tube-2 12.5 ng/mL
      Tube-3 6.25 ng/mL
      Tube-4 3.125 ng/mL
      Tube-5 1.56 ng/mL
      Tube-6 0.78 ng/mL
      Tube-7 0.39 ng/mL
      Tube-8 0 ng/mL


4. EIA buffer 230 μL

See following picture.

5) Dilution of test sample
   Test samples need to be diluted with “4, EIA buffer” accordingly.

If the concentration of human sAPPβ-sw 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-8) 100 μL

Incubation overnight at 4°C with plate lid

4 times (wash buffer more than 350 μL)*

Labeled Antibody 100 μL 100 μL 100 μL

- Incubation for 30 minutes at 4°C with plate lid

5 times (wash buffer more than 350 μL)*

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 dilutions of standard (tube-1-7) into the appropriate wells.

3) Incubate the preassayed 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 preassayed 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 of “6, Chromogen” into 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 preassayed plate for 30 minutes at room temperature in the dark.

The liquid will turn blue by addition of “6, Chromogen”.

10) Pipette 100 μL of “7, Stop solution” into the wells. Mix the liquid by tapping the side of preassayed 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 preassayed plate and confirm there is no bubble on the surface of the plate. 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 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 preassayed plate. Insufficient washing may lead to the failure in measurement.

6) Remove the wash buffer completely by tapping the preassayed 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 prepared freshly.

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. Plot the subtracted absorbance of the standards against the standard concentration on log-log graph paper. 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

<table>
<thead>
<tr>
<th>Conc. (ng/mL)</th>
<th>Absorbance (450nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>2.835</td>
</tr>
<tr>
<td>12.5</td>
<td>1.605</td>
</tr>
<tr>
<td>6.25</td>
<td>0.862</td>
</tr>
<tr>
<td>3.13</td>
<td>0.449</td>
</tr>
<tr>
<td>1.56</td>
<td>0.225</td>
</tr>
<tr>
<td>0.78</td>
<td>0.111</td>
</tr>
<tr>
<td>0.39</td>
<td>0.052</td>
</tr>
</tbody>
</table>

0 (Test Sample Blank) 0.000

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>8</td>
<td>2.30</td>
<td>3.13</td>
<td>73.5</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>1.23</td>
<td>1.56</td>
<td>78.8</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>0.59</td>
<td>0.78</td>
<td>75.6</td>
</tr>
<tr>
<td>Mouse Plasma (C57BL/6N) (EDTA)</td>
<td>8</td>
<td>2.21</td>
<td>3.13</td>
<td>70.6</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>1.18</td>
<td>1.56</td>
<td>75.6</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>0.58</td>
<td>0.78</td>
<td>74.4</td>
</tr>
</tbody>
</table>

2. Added Recovery Assay

<table>
<thead>
<tr>
<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 (x16)</td>
<td>6.25</td>
<td>4.30</td>
<td>68.8</td>
</tr>
<tr>
<td></td>
<td>3.13</td>
<td>2.21</td>
<td>70.6</td>
</tr>
<tr>
<td></td>
<td>1.56</td>
<td>1.11</td>
<td>71.2</td>
</tr>
<tr>
<td>Mouse Plasma (C57BL/6N) (EDTA) (x16)</td>
<td>6.25</td>
<td>4.67</td>
<td>74.7</td>
</tr>
<tr>
<td></td>
<td>3.13</td>
<td>2.42</td>
<td>77.3</td>
</tr>
<tr>
<td></td>
<td>1.56</td>
<td>1.22</td>
<td>78.2</td>
</tr>
</tbody>
</table>

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>8.20</td>
<td>0.32</td>
<td>3.9</td>
<td>21</td>
</tr>
<tr>
<td>4.21</td>
<td>0.17</td>
<td>4.0</td>
<td>21</td>
</tr>
<tr>
<td>1.61</td>
<td>0.07</td>
<td>4.3</td>
<td>21</td>
</tr>
</tbody>
</table>

4. Inter - 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>7.75</td>
<td>0.50</td>
<td>6.5</td>
<td>7</td>
</tr>
<tr>
<td>3.91</td>
<td>0.29</td>
<td>7.4</td>
<td>7</td>
</tr>
<tr>
<td>1.49</td>
<td>0.14</td>
<td>9.4</td>
<td>7</td>
</tr>
</tbody>
</table>

5. Specificity

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human sAPPß-sw</td>
<td>100 %</td>
</tr>
<tr>
<td>Human sAPPα</td>
<td>≤0.1 %</td>
</tr>
<tr>
<td>Human sAPPß-wild type</td>
<td>0.11 %</td>
</tr>
</tbody>
</table>

6. Sensitivity

0.07 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.)