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 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 (Lys670→Asn and Meth671→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 Amyloid-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 highly 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.

MEASUREMENT RANGE
0.78 - 50 ng/mL

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

KIT COMPONENT
1) Precoated plate: Anti-Human sAPPβ-w Type Rabbit IgG Affinity Purify 96Well x 1
2) Labeled antibody Conc. 0.3 ng/mL x 1
3) Standard: Recombinant human sAPPβ-w type protein 0.5mL x 2
4) EIA buffer 30mL x 1
5) Solution for Labeled antibody 12mL x 1
6) Chromogen: TMB solution 15mL x 1
7) Stop solution 230μL x 1
8) Wash buffer Conc.* 50mL x 1

OPERATION MANUAL
1. Materials needed but not supplied
   - Plate reader (450nm)
   - Microspatula and tip
   - Graduated cylinder and beaker
   - Deionized water
   - Refrigerator (as 4°C)
   - Graph paper (log/log)
   - Tube for dilution of Standard
   - 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 (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 "8, 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, 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 800μL (Dilute 30μL of "2, Labeled antibody Conc." with 870μL of "5, Solution for Labeled antibody" and mix it. And use the resulting solution by 100μL 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 230μL each of "4, EIA buffer" into the tube. Specify the following concentration of each tube.

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.

   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.

   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.
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

<table>
<thead>
<tr>
<th>Conc. (ng/mL)</th>
<th>Absorbance (450nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>2.918</td>
</tr>
<tr>
<td>25</td>
<td>1.953</td>
</tr>
<tr>
<td>12.5</td>
<td>1.129</td>
</tr>
<tr>
<td>6.25</td>
<td>0.674</td>
</tr>
<tr>
<td>3.13</td>
<td>0.384</td>
</tr>
<tr>
<td>1.56</td>
<td>0.230</td>
</tr>
<tr>
<td>0.78</td>
<td>0.150</td>
</tr>
<tr>
<td>0.37 (Test Sample Blank)</td>
<td>0.059</td>
</tr>
</tbody>
</table>

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.)

PERCAUTION 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. "3, Standard" is lyophilized products. Be careful to open this vial.
3. "7, Stop solution" is a strong acid substance. Therefore, be careful not to have your skin and clothes contact "7, Stop solution" and pay attention to the disposal of "7, Stop solution".
4. Dispose used materials after rinsing them with large quantity of water.
5. Precipitation may occur 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 a different lot or kit.
8. Do not use expired reagents.
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 expiry date is specified on outer box.

REFERENCE


Made in Japan.

Distributed by:

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* 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.