Human beta-Amyloid (1-42) ELISA Kit ARG81143

Human beta-Amyloid (1 - 42)

ELISA Kit

Enzyme Immunoassay for the quantification of human beta-Amyloid (1 - 42) in EDTA-plasma, cerebrospinal fluid and cell culture supernatant

Catalog number: ARG81143

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

www.arigobio.com
Human beta-Amyloid (1-42) ELISA Kit ARG81143

TABLE OF CONTENTS

SECTION ........................................ Page
INTRODUCTION ................................................................. 3
PRINCIPLE OF THE ASSAY .............................................. 3
MATERIALS PROVIDED & STORAGE INFORMATION .................. 4
MATERIALS REQUIRED BUT NOT PROVIDED .......................... 4
TECHNICAL HINTS AND PRECAUTIONS ................................. 4
SAMPLE COLLECTION & STORAGE INFORMATION .................. 5
REAGENT PREPARATION ...................................................... 5
ASSAY PROCEDURE ............................................................. 7
CALCULATION OF RESULTS ............................................... 8
EXAMPLE OF TYPICAL STANDARD CURVE .............................. 9
QUALITY ASSURANCE ......................................................... 9

MANUFACTURED BY:
Arigo Biolaboratories Corporation
Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan
Phone: +886 (3) 562 1738
Fax: +886 (3) 561 3008
Email: info@arigobio.com

www.arigobio.com
INTRODUCTION

Alzheimer’s Disease (AD) is the most common neurodegenerative disorder in elderly people. It has been demonstrated that AD has biological causes and is characterized by the presence of senile plaques and neurofibrillary tangles mainly in cerebral cortex and hippocampus brain regions. Beta-Amyloid (1-40) (Aβ40) and beta-Amyloid (1-42) (Aβ42) are the main components of the above plaques; however, other forms of beta-Amyloid peptides are also present. Both peptides are cleaved from the Amyloid Precursor Protein (APP) by β-secretase and γ-secretase enzymes. Many studies suggest that Aβ42 or/and Aβ43 are required to initiate formation of amyloid plaques and neurofibrills that leads to the neurodegeneration, while Aβ40 is less neurotoxic.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for Aβ 42 has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Aβ 42 present is bound by the immobilized antibody. After washing away any unbound substances a HRP-labeled Human Aβ (N) monoclonal antibody is added to each well and incubate. After washing away any unbound reagents, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Aβ 42 bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm. The concentration of Aβ 42 in the sample is then determined by comparing the O.D of samples to the standard curve.
MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Storage information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody-coated microplate</td>
<td>8 X 12 strips</td>
<td>4°C</td>
</tr>
<tr>
<td>Standard</td>
<td>2 vial (0.5 ml)</td>
<td>4°C, lyophilized</td>
</tr>
<tr>
<td>HRP-conjugated antibody concentrate (30X)</td>
<td>0.4 ml</td>
<td>4°C</td>
</tr>
<tr>
<td>1X conjugated antibody dilution buffer</td>
<td>12 ml</td>
<td>4°C</td>
</tr>
<tr>
<td>1X Assay Buffer</td>
<td>30 ml</td>
<td>4°C</td>
</tr>
<tr>
<td>40X Wash buffer</td>
<td>50 ml</td>
<td>4°C</td>
</tr>
<tr>
<td>TMB substrate</td>
<td>15 ml</td>
<td>4°C (Protect from light)</td>
</tr>
<tr>
<td>STOP solution</td>
<td>12 ml</td>
<td>4°C</td>
</tr>
</tbody>
</table>

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the HRP-labeled detection antibody before use.
Human beta-Amyloid (1-42) ELISA Kit ARG81143

- If crystals are observed in the 10X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

**Cell Culture Supernatants** - Remove particulates by centrifugation and aliquot & store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Plasma** - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute 40X Wash buffer into distilled water to yield 1X Wash buffer (E.g.: 50 ml 40X Wash buffer + 1950 ml distilled water). The prepared wash buffer should be stored in refrigerator and used within 2 weeks after dilution.

- **1X HRP-conjugated antibody**: Dilute HRP-conjugated antibody concentrate at 1:30 dilution ratio in 1X conjugated antibody dilution buffer. (This operation should be done just before the application of HRP-conjugated antibody)
Standards: Reconstitute the standards with 0.5 ml of distilled water to yield a stock concentration of 200 pg/ml. Make sure the standard is dissolved completely before making serial dilutions. The Assay Buffer serves as zero standard (0 pg/ml) and the rest of the standard serial dilution can be diluted into Assay Buffer with the suggested amount in the brackets according to the concentration below: 100, 50, 25, 12.5, 6.25, 3.13, 1.56 pg/ml

Dilution table:

<table>
<thead>
<tr>
<th>Aβ 42 Concentration (pg/ml)</th>
<th>Dilution (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>230 µl (Assay Buffer) + 230 µl (200 pg/ml)</td>
</tr>
<tr>
<td>50</td>
<td>230 µl (Assay Buffer) + 230 µl (100 pg/ml)</td>
</tr>
<tr>
<td>25</td>
<td>230 µl (Assay Buffer) + 230 µl (50 pg/ml)</td>
</tr>
<tr>
<td>12.5</td>
<td>230 µl (Assay Buffer) + 230 µl (25 pg/ml)</td>
</tr>
<tr>
<td>6.25</td>
<td>230 µl (Assay Buffer) + 230 µl (12.5 pg/ml)</td>
</tr>
<tr>
<td>3.13</td>
<td>230 µl (Assay Buffer) + 230 µl (6.25 pg/ml)</td>
</tr>
<tr>
<td>1.56</td>
<td>230 µl (Assay Buffer) + 230 µl (3.13 pg/ml)</td>
</tr>
<tr>
<td>0</td>
<td>230 µl (Assay Buffer)</td>
</tr>
</tbody>
</table>

Samples: If the initial assay found samples contain Aβ 42 higher than the highest standard, the samples can be diluted with Assay Buffer and then re-assay the samples, so pre-assay with several different dilutions will be
Human beta-Amyloid (1-42) ELISA Kit ARG81143

recommended to determine the proper dilution of samples.
For the calculation of the concentrations this dilution factor has to be taken into account.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 μl of standards, samples and zero controls (Assay Buffer) into wells. Cover the plate and incubate for overnight at 4 °C.
3. Aspirate each well and wash, repeating the process 6 times for a total 7 washes. Wash by filling each well with 1x Wash Buffer (350 μl) using a squirt bottle, manifold dispenser, or autowasher, and keep the wash buffer in the wells for 15-30 sec. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add 100 μl 1X HRP-conjugated antibody into each well. Cover wells and incubate for 1 hour at 4°C.
5. Aspirate each well and wash, repeating the process 8 times for a total 9 washes. Wash by filling each well with 1x Wash Buffer (350 μl) using a squirt bottle, manifold dispenser, or autowasher, and keep the wash buffer in the wells for 15-30 sec. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add 100 μl of TMB Reagent to each well. Incubate for 30 minutes at RT in the dark.

7. Add 100 μl of Stop Solution to each well. The color of the solution should change from blue to yellow.

8. Read the OD with a microplate reader at 450nm immediately.

**CALCULATION OF RESULTS**

1. Calculate the average absorbance values for each set of standards, controls or samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. Refer to the table below for molar conversion:

<table>
<thead>
<tr>
<th>Standard</th>
<th>Concentration of standards</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ 42 (pg/ml)</td>
<td>0</td>
</tr>
<tr>
<td>Aβ 42 (pmol/L)</td>
<td>0</td>
</tr>
</tbody>
</table>

Conversion: Aβ 42 (pg/ml) x 0.2217 = Aβ 42 (pmol/L)
EXAMPLE OF TYPICAL STANDARD CURVE
The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

QUALITY ASSURANCE

Sensitivity
Detects as low as 0.29 pg/mL of human beta-Amyloid (1-42).

Specificity

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Cross reactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Aβ 42</td>
<td>100</td>
</tr>
<tr>
<td>Human Aβ 40</td>
<td>≤ 0.1</td>
</tr>
<tr>
<td>Human Aβ 43</td>
<td>&lt; 0.8</td>
</tr>
<tr>
<td>Mouse/Rat Aβ (1-42)</td>
<td>10 -40</td>
</tr>
</tbody>
</table>

Intra-assay precision
The CV value of intra-assay and inter-assay precision was 4.3 - 13%.

Recovery

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human EDTA-plasma</td>
<td>84 - 89</td>
</tr>
<tr>
<td>Human cerebrospinal fluid</td>
<td>103 - 115</td>
</tr>
<tr>
<td>Human cell culture supernatant</td>
<td>106 - 118</td>
</tr>
<tr>
<td>(RPMI1640 with 10% FCS)</td>
<td></td>
</tr>
</tbody>
</table>
Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.