Mouse/Rat beta-Amyloid (1 - 42) ELISA Kit

Enzyme Immunoassay for the quantification of mouse/rat beta-Amyloid (1 - 42) in serum and plasma (EDTA)

Catalog number: ARG80981

distributed in the US/Canada by:
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For research use only. Not for use in diagnostic procedures.

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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 mouse/rat Aβ (38-42) has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells. After washing away any unbound substances, the HRP-labeled mouse/rat Aβ (1-16) antibody is added to each well and incubate. Following a washing to remove unbound substances, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Aβ 1-42 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 1-42 in the sample is then determined by comparing the

O.D of samples to the standard curve. The concentration of antigen is directly proportional to the optical density measured in the wells.

MATERIALS PROVIDED & STORAGE INFORMATION

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

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C
Standard: Mouse/Rat Aβ (1-42).	2 vial	4°C, lyophilized
HRP-labeled detection antibody (30X)	400 µl	4°C
Assay Buffer	30 ml (Ready to use)	4°C
Conjugate antibody dilution buffer	12 ml (Ready to use)	4°C
40X Wash buffer	50 ml	4°C
TMB substrate	15 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	12 ml (Ready-to-use)	4°C

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.
- If crystals are observed in the 40X 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.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and 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.

Note: protease inhibitor cocktail with PMSF must be added to all samples to

avoid protein degradation.

100X Protease Inhibitor Cocktail

a. Aprotinin 0.4 mg, Leupeptin 2 mg, ddH_2O 900 µl

b. Dissolve 0.1 mg of Pepstatin A in 100 µl of methanol

Mix a. and b., aliquot and store cocktail at –80 °C.

100X 100 mM PMSF Solution

Dissolve 174 mg of PMSF in 10 ml of pure isopropanol.

Aliquot and store at –80 °C.

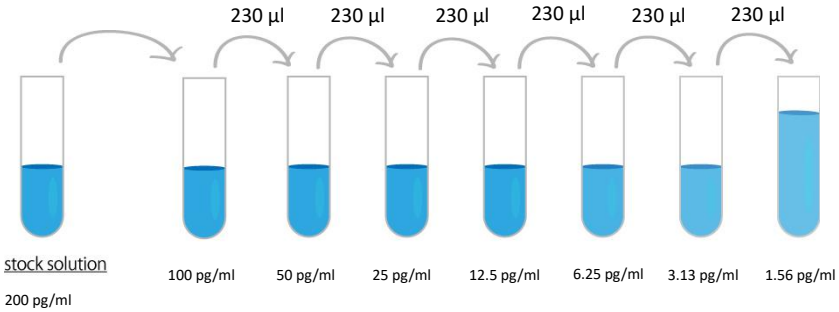
Add to the protease inhibitor cocktail (1mM final concentration) immediately before use.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute 40X Wash buffer into distilled water to yield 1X Wash buffer.
- **1X HRP-labeled detection antibody:** Dilute 30X HRP-labeled detection antibody into Conjugate antibody dilution buffer to yield 1X HRP-labeled detection antibody.

  This operation should be done just before the application of adding Enzyme Conjugate antibody. The remaining 30X HRP-labeled detection antibody should be stored at 4°C in firmly sealed vial.
- **Sample:** If the concentration of Mouse/Rat Aβ (1-42) 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. Test sample may be diluted with Assay Buffer as necessary.

- **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). The rest of the standard serial dilution can be diluted into Assay Buffer as according to the suggested concentration below: 100, 50, 25, 12.5, 6.25, 3.13, 1.56 pg/ml



Standard	Mouse/Rat Aβ (1-42) (pg/ml)	Dilution
S1	100	230 µl assay buffer + 230 µl (stock)
S2	50	230 µl assay buffer + 230 µl (S1)
S3	25	230 µl assay buffer + 230 µl (S2)
S4	12.5	230 µl assay buffer + 230 µl (S3)
S5	6.25	230 µl assay buffer + 230 µl (S4)
S6	3.13	230 µl assay buffer + 230 µl (S5)
S7	1.56	230 µl assay buffer + 230 µl (S6)
S8	0	230 µl assay buffer as zero standard

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) approximately 30 minutes 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 (S0-S8) and samples in duplicates into wells. Add 100 µl Assay Buffer in the other wells as reagent blank.
3. Cover the plate and incubate the plate at 4°C for overnight.
4. Aspirate each well and wash, repeating the process 6 times for a total 7 washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Keep the wash buffer in the wells for 15-30 seconds before remove it. Complete removal of liquid at each wash step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add 100 µl of 1X HRP-labeled detection antibody into each well (except reagent blank), and incubate the plate at 4°C for 60 min in dark.
6. Aspirate each well and wash, repeating the process 5 times for a total 6 washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Keep the wash buffer in the wells for 15-30 seconds before remove it. Complete removal of liquid at each wash step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting

against clean paper towels.

7. Add 100 µl of TMB Reagent to each well. Incubate for 10-15 minutes at room temperature in dark.
8. Add 50 µl of Stop Solution to each well. The color of the solution should change from blue to yellow.
9. 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.

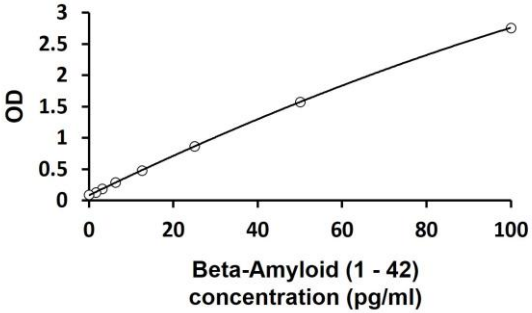
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

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.

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

0.05 pg/ml.

Assay range: 1.56 - 100 pg/mL

(0.35 – 22.6 pmol/L, as molecular weight of Aβ (1-42) is 4418.0)

Specificity

Peptide	Cross reactivity, %
Mouse/Rat Aβ 1–42	100
Mouse/Rat Aβ 1–40	< 0.1
Human Aβ 1–42	1.3

Intra-assay precision

The CV value of intra-assay and inter-assay precision was 5.8%.

Recovery

51.1-83.6%

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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.

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