Mouse/Rat beta-Amyloid (1 - 40) ELISA Kit

Enzyme Immunoassay for the quantification of Mouse/Rat beta-Amyloid (1 - 40) in brain extract, cell culture supernatants, serum and plasma.

Catalog number: ARG80980

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

The first case of Alzheimer's disease was defined and reported in 1907 by the German scientist, Dr. A. Alzheimer. His studies have shown that this is the main cause of dementia in the elderly. The plaques which appear in the brains of Alzheimer's disease patients are mostly constituted by the Amyloid beta protein (A-beta). A-beta is a peptide which consists of 40 or 42 (43) amino acids, and reports show that this is cleaved by beta- and gamma-secretase from the amyloid precursor protein.

In addition, the presence of numerous variant A-beta molecules has been demonstrated in the culture fluid of mouse neuroblastoma cells transfected with cDNA coding human amyloid precursor protein (APP).

Furthermore, in 1995, a dominant and differential deposition of distinct beta amyloid peptide species, A-beta (N3pE), in senile plaques was found by Saido et al. This modified molecule, starting at the 3rd amino terminal residue, glutamate, was discovered to convert to pyroglutamate through intramolecular dehydration.

PRINCIPLE OF THE ASSAY

This ELISA kit is capable for measuring mouse and rat beta-Amyloid (1 - 40), it does not detect p3 peptide, which is cleaved by alpha or gamma secretase.

This product is also useful for measuring mouse and rat derived beta-Amyloid in brain extract, cell culture supernatant and blood sample (serum or plasma) of mouse or rat.

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for beta-Amyloid (35 - 40) has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells, and any beta-Amyloid (1 - 40) present is bound by the immobilized antibody. After washing away any unbound substances, a HRP-conjugated antibody specific for mouse/rat beta-Amyloid (1 - 16) is added to each well and incubate. After incubation, the capture antibody-antigen complex is developed and immobilized to the pre-coated wells. After washing away any unbound substances, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of beta-Amyloid (1 - 40) 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 beta-Amyloid (1 - 40) 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	2 vials	4°C, lyophilized
30X HRP-labeled antibody	1 X 400 µl	4°C
Conjugated ab Diluent Buffer	1 X 12 ml (Ready-to-use)	4°C
Assay Diluent	1 X 30 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 antibody conjugate concentrate and HRP-Streptavidin concentrate 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.

Cell Culture Supernatants - Remove particulates by centrifugation and aliquot & store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Dilute cell culture supernatant in an appropriate dilution ratio with Assay Buffer and use as a sample

Note: If the culture supernatant contains FCS, Aβ (1-40)-like substances might be measured, therefore, it is recommended that a negative control should be set.

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 or heparin 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: Dilute serum or plasma in an appropriate dilution ratio with Assay Buffer, and use as a sample.

Brain extracted solution - Add 5 volumes of extraction buffer (1% CHAPS in TBS pH7.6) to brain sample, and homogenize them.

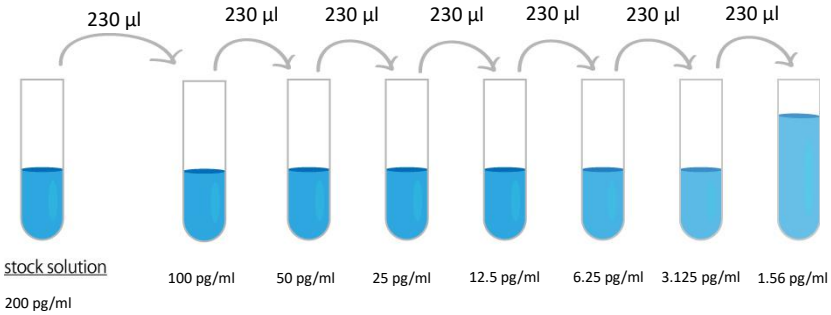
After thorough homogenization, let stand the emulsion on ice for at least 3 hours. Centrifuge it at 70,000 rpm for 20 minutes at 4°C, and then appropriately dilute the supernatant with Assay Buffer and use for

measurement.

Note: when using brain extracted solution adjusted with extraction buffer, using the same extraction buffer for standard reconstruction instead of deionized water.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 40X Wash buffer into deionized water to yield 1X Wash buffer.
- 1X HRP-labeled antibody: Dilute 1:30 with Conjugated ab Diluent Buffer. This operation should be done just before adding 1X HRP-labeled antibody and only preparing the needed volume for once. And the remaining 30X HRP-labeled antibody should be stored at 4°C in firmly sealed vial.
- Standards: Reconstitute the standards with 0.5 ml deionized water to yield a stock concentration of 200 pg/ml. For measuring brain extract samples use the same extraction buffer for standard reconstruction instead of deionized water. Make sure the standard is dissolved completely before making serial dilutions. The Assay Diluent serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted into Assay diluent buffer as according to the suggested concentration below: 100, 50, 25, 12.5, 6.25, 3.125 and 1.56 pg/ml



The example of the dilution of standards

Standard (pg/ml)	Standard (pmol/L)	Buffer of Dilution
100	23.6	200 pg/ml (230 µl) + Assay Diluent (230 µl)
50	11.8	100 pg/ml (230 µl) + Assay Diluent (230 µl)
25	5.9	50 pg/ml (230 µl) + Assay Diluent (230 µl)
12.5	2.95	25 pg/ml (230 µl) + Assay Diluent (230 µl)
6.25	1.475	12.5 pg/ml (230 µl) + Assay Diluent (230 µl)
3.125	0.736	5.25 pg/ml (230 µl) + Assay Diluent (230 µl)
1.56	0.368	3.125 pg/ml (230 µl) + Assay Diluent (230 µl)
0	0	Assay Diluent (230 µl)

- **Samples**: If the measuring absorbance of samples is higher than the highest standard, dilute the samples with Assay Diluent before assay and assay again.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) 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 (0-100 pg/ml) and samples into wells (Assay Diluent serves as zero standard). Add 100 µl Assay Diluent into additional two wells as blank. Cover with enclosed foil, 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 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher and keep the wash buffer in the well for 15-30 seconds. 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 of 1X HRP-labeled antibody solution to each well (except blank). Cover wells and incubate for 1h at 4°C.
5. Aspirate each well and wash the microtiter plate 9 times as step 3.
6. Add 100 µl of TMB substrate to each well. Incubate for 30 minutes at room temperature in dark. (Take the required quantity of TMB substrate into a disposable test tube before use, and do not return the rest of the test tube to original TMB substrate bottle to avoid contamination.) The liquid will become blue by addition of TMB Substrate Solution.
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 450 nm immediately. The

measurement should be done within 30 minutes after addition of STOP Solution.

CALCULATION OF RESULTS

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

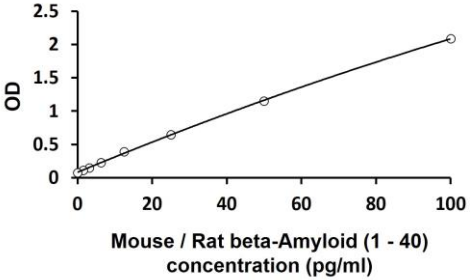
2. Using linear or log-log graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

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

The minimum detectable dose (MDD) of Mouse/Rat beta-Amyloid (1 - 40) was 0.28 pg/ml

Specificity

Compound	Cross Reactivity
Mouse/Rat Aβ (1-40)	100%
Human Aβ(1-40)	0.8%
Mouse Aβ (1-42)	0.2%

Intra-assay and Inter-assay precision

The CV value of intra-assay was < 5% and inter-assay precision was < 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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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.