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

Enzyme Immunoassay for the quantification of mouse/rat beta-Amyloid (1 - 42) in brain lysate, cerebrospinal fluid and plasma

Catalog number: ARG80939

Package: 96 wells

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 Aβ 42 has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells together with HRP-labeled Aβ 42 antibody. After incubation with continuous agitation, the capture antibody-antigen-detection antibody complex is developed and immobilized to the pre-coated wells. 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. 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	1 μg (1 vial)	4°C
Peptide Standard Reconstitution Buffer	1 ml (Ready-to-use)	4°C
HRP-labeled detection antibody	50 μl	4°C
1X Detection antibody Dilution Buffer	10 ml (Ready-to-use)	4°C
1X Sample Dilution Buffer	60 ml (Ready-to-use)	4°C
10X Wash buffer	30 ml	4°C
TMB substrate	10 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	10 ml (Ready-to-use)	4°C
Cover sticks	2 Pieces	

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 10X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Bring all reagents to room temperature (20- 25°C) before use.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- 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.

Note: protease inhibitor cocktail with PMSF must be added to all samples to avoid protein degradation.

Brain lysate- Mix 800 µl of homogenate buffer (5M Guanidine HCl, 50 mM Tris HCl pH=8.0) with 100 mg of brain sample in a Dounce homogenizer placed on ice. Homogenize the tissue thoroughly and incubate at room temperature for 3-4 hours with mixing. Guanidine-HCl brain homogenates are stable at 4°C for several weeks and can be freeze-thawed many times without degradation of beta-Amyloid peptides.

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.

Cerebrospinal fluid (CSF)- Remove particulates by centrifugation at 2000 X g for 10 min. Collect supernatants and assay immediately or aliquot and store samples at ≤ -80°C. Avoid repeated freeze-thaw cycles.

100X Protease Inhibitor Cocktail

a. Dissolve 0.4 mg of Aprotinin and 2 mg of Leupeptin in 900 µl of ddH_2O.

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

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

100 mM PMSF Solution (100X)

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

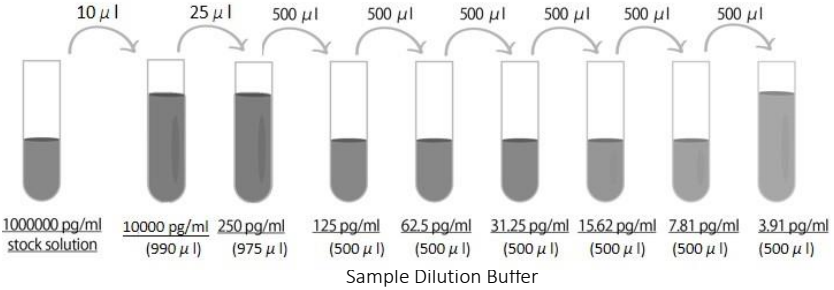
Aliquot and store at –80 °C.

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

REAGENT PREPARATION

- **1X Wash buffer**: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer.
- **1X HRP-labeled detection antibody:** Dilute detection antibody 200x dilution with detection antibody dilution buffer. Please only prepare amount needed.
- **Sample:** Diluting plasma at 1:20 dilution ratio and CSF at 1:4 dilution ratio with 1X Sample Dilution Buffer. For brain lysate, it's recommended to start with 1:40 dilution ratio with 1X Sample Dilution Buffer **(Guanidine-HCl concentration higher than 0.125M will result in a significant loss of signal in the assay!)**. User should determine the optimal dilution factor. Protease inhibitor cocktail with PMSF must be added to all samples to avoid protein degradation. Transgenic mouse brain lysates might require higher dilution factor due to the high concentration of Aβ42 peptide present in the brain.
- **Standards:** Reconstitute the standards with 1 ml of Peptide Standard Reconstitute Buffer (1,000,000 pg/ml). Keep the buffer in the vial for 10 minutes and mix and then gently mix (no foam should result) to dissolve completely. Make sure the standard is dissolved completely before making serial dilutions. Reconstituted Peptide Standard must be stored at -80 °C in

small aliquots (100 µl/vial) and avoided repeated freeze-thaw cycles. (The reconstituted Peptide Standard can only be thawed once, so do not reuse reconstituted standard once it is thawed.) The 1X Sample Dilution Buffer serves as zero standard (0 pg/ml). The rest of the standard serial dilution can be diluted into Sample Dilution Buffer as according to the suggested concentration below: 250, 125, 62.5, 31.25, 15.625, 7.8125, 3.91 pg/ml



Dilute standard as according to the table below:

Standard	Aβ42 Conc. (pg/ml)	µl of Sample Dilution Buffer	µl of standard
Stock	1,000,000	-	1000
High concentration	10,000	990	10 (1,000,000 pg/ml stock)
S7	250	975	25 (10,000 pg/ml standard)
S6	125	500	500 (S7)
S5	62.5	500	500 (S6)
S4	31.25	500	500 (S5)
S3	15.625	500	500 (S4)
S2	7.8125	500	500 (S3)
S1	3.91	500	500 (S2)
S0	0	500	0

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** in duplicates including blank and **100 µl** of **diluted samples** into wells.
3. Add **50 µl** of **diluted HRP-labeled detection antibody** into each well, cover the plate and incubate the plate **overnight at 4°C in dark**.
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 20 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 **TMB substrate reagent** to each well and tap the plate gently to mix the reagent. Incubate for **5-15 minutes at room temperature in dark**. (It may be necessary to adjust color development time so that absorbance values fall within the detection range.)
6. Add **50 µl** of **Stop Solution** to each well and tap the plate gently to mix the reagent. The color of the solution should change from blue to yellow.
7. Read the OD with a microplate reader at **450nm** immediately. It is recommended reading the plate within 20 min after adding the stop solution.

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.

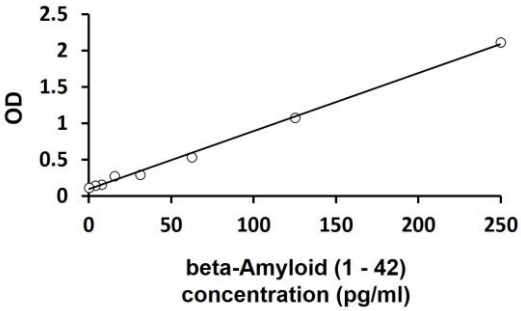
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. The concentration of the samples can be determined directly from this calibrator curve. Calculated concentrations must be multiplied by the sample dilution factor. (Please refer the sample preparation section as above).

6. Samples with concentrations higher than that of the highest calibrator have to be further diluted. For the calculation of the concentrations, this dilution factor has to be taken into account.

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 2 pg/ml (9 fmoles/ml) of mouse/rat beta-Amyloid (1-42) in brain lysate.

Specificity

Peptide (125 pg/ml)	**Cross reactivity**	**Peptide** (125 pg/ml)	**Cross reactivity**
Mouse/Rat Aβ 42	100 %	Mouse/Rat Aβ 37	<0.1 %
Mouse/Rat Aβ 40	<5 %	Mouse/Rat Aβ 28	<0.1 %

Intra-assay precision

The CV value of intra-assay and inter-assay precision was 4.25%.

Recovery

Specimen	Theoretical Value	Measured Value	Recovery
Brain lysate	10 pg/ml	9.4 pg/ml	94 %
Brain lysate	35 pg/ml	33.6 pg/ml	96 %