BioVendor Group

ENG

Product Data Sheet:

MOUSE AMYLOID BETA 40 ELISA

Catalogue number:
RIG013R

For research use only!

BioVendor R&D

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1. INTENDED USE

Mouse Amyloid beta 40ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of mouse Aβ40 in tissue culture supernatant, tissue homogenate, and cerebrospinal fluid [CSF]. The assay recognizes both natural and recombinant mouse Aβ40, and does not detect Aβ42 or Aβ43.

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Note: For safety and biohazard guidelines read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

2. REAGENTS PROVIDED

Antibody-Coated Wells; 96-well plate

1 vial (11 ml) Mouse Amyloid beta 40 **Detection Antibody**; contains 15 0.1% sodium azide; blue dye [1]
1 vial (0.125 ml) **Anti-Rabbit IgG HRP (100X)**
1 vial **Mouse Amyloid beta 40 Standard,** lyophilized; contains 0.1% sodium azide; refer to the Quality Control Sheet for quantity and reconstitution volume
1 vial (25 ml) **HRP Diluent;** contains 3.3 mM thymol, yellow dye [1]
1 vial (60 ml) **Standard Diluent Buffer;** contains 0.1% sodium azide, red dye [1]
1 bottle (100 ml) **Wash Buffer Concentrate (25X)**
1 vial (25 ml) **Stabilized Chromogen, Tetramethylbenzidine (TMB)**
1 vial (25 ml) **Stop Solution**
2 **Adhesive Films**

[1] To avoid pipetting mistakes, colored Standard Diluent Buffer, Detection Antibody, and HRP Diluent are provided to monitor the addition of solution to each well. Dyes do not interfere with test results.

3. STORAGE INSTRUCTIONS – ELISA KIT

Upon receipt, store the kit at 2°C to 8°C.

4. MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled or deionized water
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- 4-(2-aminoethyl) benzenesulfonyl fluoride (AEBSF) or protease inhibitor cocktail containing AEBSF
- Standard Reconstitution Buffer (55 mM Sodium Bicarbonate Buffer [NaHCO3, ultrapure grade], pH 9.0)

5. PRECAUTIONS FOR USE

- IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

6. PREPARATION OF REAGENTS

6.1 Preparation of 1x Wash Buffer

1. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

6.2 Preparation standard reconstitution buffer

1. Dissolve 2.31 g sodium bicarbonate in 500 mL deionized water.
2. Add 2 N sodium hydroxide until pH is 9.0.
3. Filter solution through a 0.2 µM filter unit.

7. PREPARATION OF SAMPLES

Prepare Brain homogenate

Note: See the below for detailed information on preparing brain homogenates.

1. Weigh out ~100 mg (wet mass) of mouse brain sample in a microcentrifuge tube.
2. Add 8 × the brain mass of cold 5 M guanidine-HCl/50 mM Tris by 50−100 µL aliquots. Homogenize thoroughly after each addition.
3. Mix the homogenate at room temperature for 3−4 hours.
4. Dilute the sample with cold BSAT-PBS Reaction Buffer with 1X protease inhibitor cocktail.

   The optimal dilution factor should be determined for each experiment, and varies depending on the quantity of Aβ present and on the inhibition of standard curve development due to the presence of guanidine. Typically, standard curve depression occurs with guanidine concentrations >0.1 M

5. Centrifuge at 16,000 × g for 20 minutes at 4°C.
6. Transfer the supernatant into clean microcentrifuge tubes and keep on ice, or store at −80°C.

Prepare Serum

1. Collect whole blood in a covered test tube.
2. After collecting whole blood, allow the blood to clot by leaving itnundisturbed at room temperature (usually 15 –30 minutes).
3. Remove the clot by centrifuging at 1000 –2000 × g for 10 minutes in a refrigerated centrifuge.
4. The resulting supernatant is serum. Immediately transfer the serum into a clean polypropylene tube using a Pasteur pipette.
5. Maintain the samples at 2 to 8°C while handling. If the serum is not analyzed immediately, aliquot the serum into 0.5 mL aliquots. Store and transport the serum aliquots at –20°C or lower.

Note: Avoid freeze-thaw cycles because this is detrimental to many serum components.

Pre-diluted samples

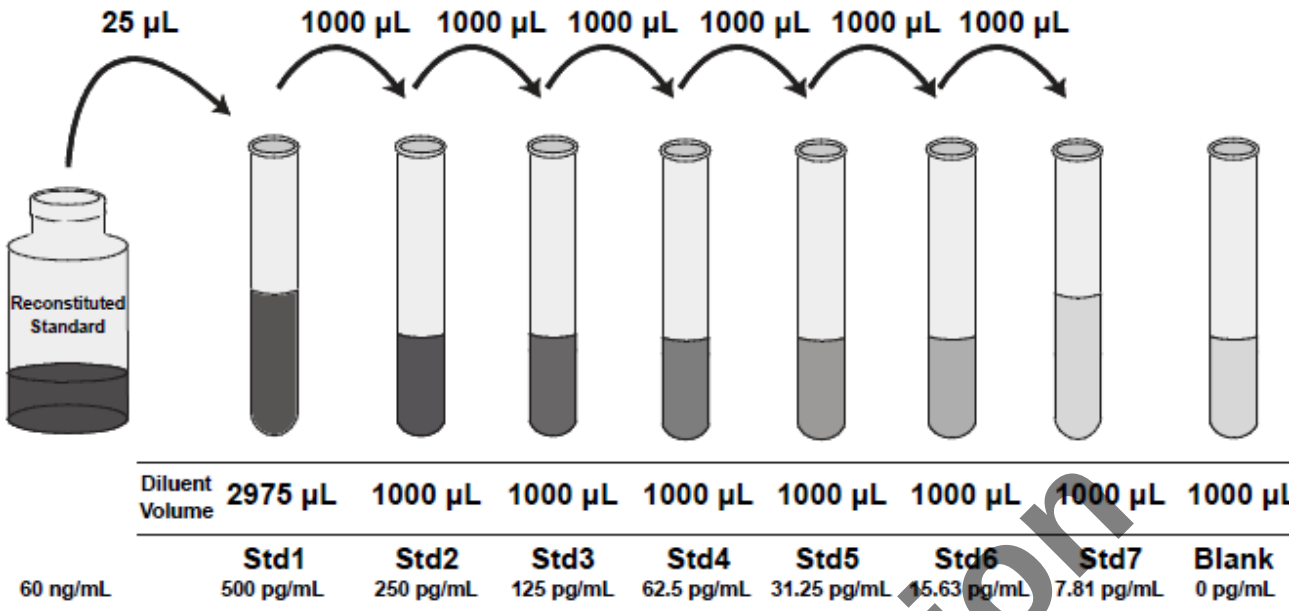
Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

- Perform sample dilutions (e.g., anywhere from 1:2 to 1:10) with Standard Diluent Buffer.
- Add AEBSF or a protease inhibitor cocktail with AEBSF to diluted samples at a final concentration of 1 mM to prevent serine proteases from degrading Aβ peptides.

Diluted Standards

Note: Use plastic tubes for diluting standards. Do not use glass tubes.

1. Reconstitute Ms Aβ40 Standard to 60 ng/mL with Standard Reconstitution Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 5 minutes to ensure complete reconstitution. Label as 60 ng/mL mouse Aβ40. Vortex briefly prior to preparing standards.
2. Add 25 µL reconstituted standard to one tube containing 2.975 mL Standard Diluent Buffer and mix. Label as 500 pg/mL mouse Aβ40.
3. Add 1 mL Standard Diluent Buffer to each of 7 tubes labeled as follows: 250, 125, 62.5, 31.25, 15.63, 7.81, and 0 pg/mL mouse Aβ40.
4. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
5. Add AEBSF or a protease inhibitor cocktail with AEBSF to the standard dilutions at a final concentration of 1 mM.
6. Remaining reconstituted standard should be discarded or frozen in aliquots at –80°C for further use. Standard can be frozen and thawed one time only without loss of immunoreactivity.



Prepare 1X Anti-Rabbit IgG HRP solution

Note: Prepare 1X Anti-Rabbit IgG HRP solution within 15 minutes of usage.

1. For each 8-well strip used in the assay, pipet 10 µL Anti-Rabbit IgG HRP (100X) solution, and dispense the solution into a tube containing 1 mL of HRP Diluent. Mix thoroughly.
2. Return the unused Anti-Rabbit IgG HRP (100X) solution to the refrigerator.

8. TEST PROTOCOL

- IMPORTANT! Perform a standard curve with each assay.
- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.

Bind antigen

a) Add 100 µL of standards, controls, or samples (see “Pre-dilute samples”on page 10) to the appropriate wells. Leave the wells for chromogen blanks empty.
b) Cover the plate with a plate cover and incubate 2 hours at room temperature.
c) Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

Add detector antibody

a) Add 100 µL of Ms Aβ40 Detection Antibody solution into each well except the chromogen blanks.
b) Cover the plate with a plate cover and incubate 1 hour at room temperature.
c) Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

Add IgG-HRP

a) Add 100 µL Anti-Rabbit IgG HRP into each well except the chromogen blanks.
b) Cover the plate with plate cover and incubate for 30 minutes at room temperature.
c) Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

Add Stabilized Chromogen

a) Add 100 µL Stabilized Chromogen to each well. The substrate solution begins to turn blue.
b) Incubate for 30 minutes at room temperature in the dark.

Note: TMB should not touch aluminum foil or other metals.

Add Stop Solution

Add 100 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

9. CALCULATION OF RESULTS

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A 4 parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

Note: Dilute samples producing signals greater than the upper limit of the standard curve in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

10. PERFORMANCE CHARACTERISTIC

Standard curve (example)

The following data were obtained for the various standards over the range of 0 to 500 pg/mL mouse Aβ40.

Standard Mouse Amyloid beta 40 (pg/mL)	Optical Density (450 nm)
500	3.69
250	1.99
125	0.77
62.5	0.36
31.25	0.19
15.63	0.12
7.81	0.10
0	0.07

Inter-assay precision

Samples were assayed 36 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	384.6	187.7	90.0
Standard Deviation	13.6	5.8	6.9
% Coefficient of Variation	3.5	3.1	7.7

Intra-assay precision

Samples of known Mouse Amyloid beta 40concentrations were assayed in replicates of 14 to determine precision within assays

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	378.3	190.9	91.8
Standard Deviation	13.3	6.1	7.6
% Coefficient of Variation	3.5	3.2	8.3

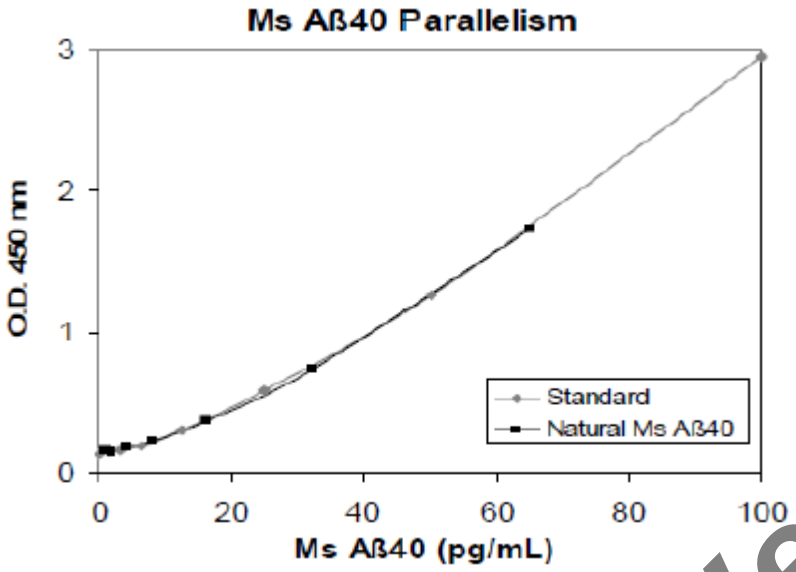
Linearity of dilution

Tissue culture supernatant from a Neuro-2a cell culture was serially diluted in Standard Diluent Buffer over the range of the assay and measured for mouse Aβ40 content. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

Dilution	Measured (pg/mL)	Expected	
		(pg/mL)	%
Neat	64.9	64.9	100.0
1/2	31.3	32.5	96.4
1/4	15.8	16.2	97.4
1/8	7.8	8.1	95.5

Paralelism

Natural mouse Aβ40 was spiked into Standard Diluent Buffer and measured against the standard used in this kit. The standard accurately reflects mouse Aβ40 content in samples.



Recovery

The recovery of mouse Aβ40 added to CSF and cell culture medium containing 10% fetal bovine serum (FBS) was measured with the Mouse Amyloid beta 40 ELISA Kit.

Sample	Average % Recovery
CSF	83
Cell culture medium (10% FBS)	120

Sensitivity

The analytical sensitivity of the assay is <5 pg/mL Mouse Amyloid beta 40. This was determined by adding two standard deviations to the mean absorbance obtained when the zero standard was assayed 30 times.

Specificity

A buffered solution of human Aβ 1 - 40 at 10 ng/mL was assayed in the Mouse Amyloid beta 40 ELISA Kit and found to have no cross-reactivity.

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