Mouse Aß42 ELISA Kit

Catalog Number KMB3441 (96 tests)

Pub. No. MAN0014858 **Rev.** 4.0 (31)

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, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Invitrogen™ Mouse Aβ42 ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of mouse Aβ42 in samples (e.g., tissue culture supernatant, tissue homogenate, cerebrospinal fluid (CSF)). The assay will recognize both natural and synthetic forms of mouse and rat Aβ42.

Contents and storage

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

Contents	Cat. No. KMB3441 (96 tests)
Ms Aß42 Standard, lyophilized synthetic peptide; contains 0.1% sodium azide	1 vial
Standard Diluent Buffer; contains 0.1% sodium azide, red dye [1]	25 mL
Antibody Coated Wells. 96-well plate	1 plate
Ms Aß42 Detection Antibody; contains 0.1% sodium azide, blue dye [1]	11 mL
Anti-Rabbit IgG HRP (100X)	0.125 mL
HRP Diluent; contains 3.3 mM thymol, yellow dye [1]	25 mL
Wash Buffer Concentrate (25X)	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Adhesive Plate Covers	2

[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.

Materials required but not provided

- Standard Reconstitution Buffer (55 mM Sodium Bicarbonate Buffer [$NaHCO_3$, ultrapure grade], pH 9.0)
- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents
- Orbital microplate shaker set to approximately 100 rpm
- 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF) or protease inhibitor cocktail containing AEBSF

Before you begin

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Prepare 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.

Prepare 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.

Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.
- When analyzing samples, add a protease inhibitor cocktail with AEBSF (a serine protease inhibitor) and prepare the standard dilutions using the same diluent as used with the biological samples. Serine proteases can rapidly degrade Aβ peptides, thus using AEBSF (water soluble andless toxic than PMSF) at a 1 mM final concentration is helpful. Keep samples on ice until ready to apply to plate.

Prepare brain homogenate

Note: See the *ELISA Technical Guide* 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, pH 8.0 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 ten-fold with cold PBS with 1X protease inhibitor cocktail (e.g., Sigma Cat. No. P-2714).
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.

Pre-dilute 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.

- Samples >200pg/ml should be diluted with Standard Diluent Buffer.
- It is recommended to perform a 1:2 to 1: 10 dilution of samples for analyzing Aβ42. This dilution must be performed because certain components in samples can interfere with the detection of the Aβ peptides or to bring the levels of Aβ within the range of this assay. AEBSF should be added to the diluted samples at a final concentration of 1mM in order to prevent proteolysis of the Aβ peptides.

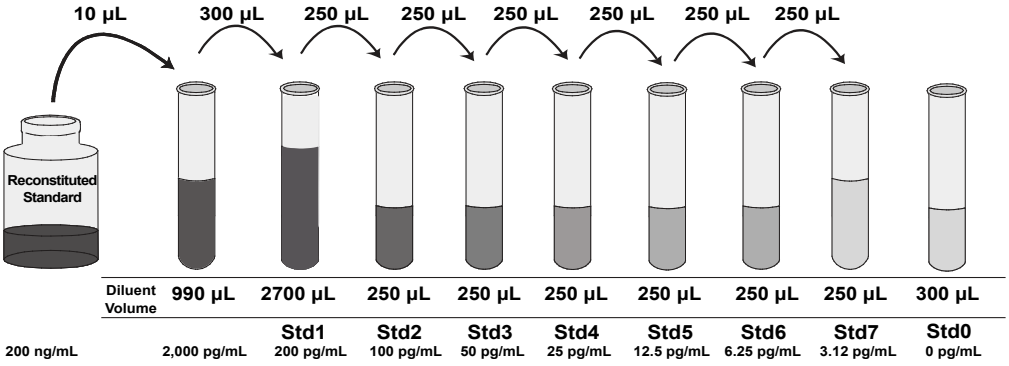
Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Note: Polypropylene tubes may be used for standard dilutions. Ms Aβ42 Standard is calibrated against highly purified mouse Aβ42 where mass was corrected for peptide content by amino acid analysis.

Note: Standard curve generation using the Aβ peptide standard must be performed using the same composition of buffers used for the diluted experimental samples. For example, if brain extracts are diluted 1:10 with Standard Diluent Buffer, then the buffer used to dilute standards should be 90% Standard Diluent Buffer and 10% brain extraction buffer (including AEBSF at a final concentration of 1 mM).

1. Reconstitute Ms Aβ42 Standard to 200 ng/mL with Standard Reconstitution Buffer (55mM sodium bicarbonate, pH 9.0). Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 200 ng/mL mouse Aβ42. **Use the standard within 1 hour of reconstitution.**
2. Add 10 µL Reconstituted Standard to one tube containing 990 µL Standard Diluent Buffer and mix. Label as 2,000 pg/mL mouse Aβ42.
3. Add 300 µL of 2,000 pg/mL standard to one tube containing 2,700 µL Standard Diluent Buffer and mix. Label as 200 pg/mL mouse Aβ42.
4. Add 250 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 100, 50, 25, 12.5, 6.25, 3.12, and 0 pg/mL mouse Aβ42.
5. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
6. Remaining reconstituted standard should be discarded or frozen in aliquots at –80°C for up to 4 months. Standard can be frozen and thawed one time only without loss of immunoreactivity. Return Standard Diluent Buffer to the refrigerator.



Prepare 1X Anti-Rabbit IgG HRP solution

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

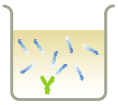
1. For each 8-well strip used in the assay, pipet 10 µL Anti-Rabbit IgG HRP (100X) solution, and dispense the solution into a tube containing 1 mL of HRP Diluent. Mix thoroughly.
2. Return the unused Anti-Rabbit IgG HRP (100X) solution to the refrigerator.

Perform ELISA (Total assay time: 4 hours)

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.

1 Bind antigen



a. Add 100 µL of standards, controls, or samples (see "Pre-dilute samples"on page 2) 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.

2 Add detector antibody

a. Add 100 µL of Ms Aβ42 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.

3 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.

4 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.

5 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.

Read the plate and generate the standard curve

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.

Performance characteristics

Standard curve example

The following data were obtained for the various standards over the range of 0 to 200 pg/mL mouse Aβ42.

Standard Mouse Aß42 (pg/mL)	Optical Density (450 nm)
200	3.27
100	2.34
50	1.33
25	0.85
12.5	0.50
6.25	0.37
3.12	0.32
0	0.23

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)	103.6	51.0	14.7
Standard Deviation	5.0	3.9	1.3
% Coefficient of Variation	4.8	7.6	8.8

Intra-assay precision

Samples of known mouse Aβ42 concentration were assayed in replicates of 12 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	103.0	47.6	14.2
Standard Deviation	6.0	1.4	1.2
% Coefficient of Variation	5.8	4.0	8.0

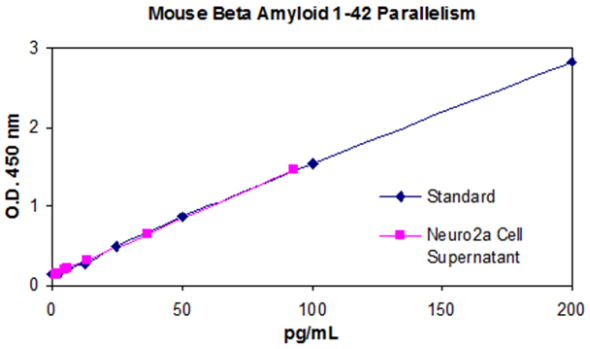
Linearity of dilution

Cell 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 Aβ42 content. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

Dilution	Cell Culture Supernatant		
	Measured (pg/mL)	Expected	
		(pg/mL)	%
1/2	36.6	36.6	100
1/4	13.4	18.3	73
1/8	6.2	9.2	67.3
1/16	4.7	4.6	103
1/32	1.8	2.3	77

Parallelism

Natural mouse Aβ42 was spiked into *Standard Diluent Buffer* and measured against the standard used in this kit. The standard accurately reflects mouse Aβ42 content in samples.



Recovery

The recovery of mouse Aβ42 added to human cerebral spinal fluid (CSF), mouse serum and plasma averaged 125%, 68% and 77%, respectively. The recovery of mouse Aβ42 added to tissue culture medium containing 10% fetal calf serum averaged 78%.

Sensitivity

The analytical sensitivity of this assay is <3 pg/mL mouse Aβ42. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times, and calculating the corresponding concentration.

Specificity

Buffered solutions of mouse Aβ42 1-40 (240 ng/mL), human APP (250 ng/mL), human Aβ (1-40) (100 ng/mL), and human Aβ (1-42) (100 ng/mL) were assayed with the Mouse β Amyloid 1-42 kit and found to have no cross-reactivity. Only Mouse Aβ42 ELISA Kit was able to be detected in this assay.

Limited product warranty

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Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

Manufacturer's address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

The information in this guide is subject to change without notice.