BioVendor Group

ENG

Product Data Sheet:

Human Amyloid beta 42 Ultrasensitive ELISA

Catalogue number:

RIG017R

For research use only!

BioVendor R&D

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1. INTENDED USE

Human Amyloid beta 42 Ultrasensitive ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of human Aβ42 (β amyloid1-42) in tissue culture medium, tissue homogenates, cerebrospinal fluid (CSF), and other sample types. The assay will recognize both natural and recombinant human Aβ42. The anti-human Aβ42 antibody used in this kit is capable of selectively detecting Aβ42 and not Aβ40/Aβ43. Alzheimer's Disease (AD) is characterized by the presence of extracellular plaques and intracellular neurofibrillary tangles (NFTs) in the brain. The

major protein component of these plaques is β amyloid peptide (Aβ), a 40 to 43 amino acid peptide cleaved from amyloid precursor protein by β-secretase (BACE) and a putative γ (gamma) secretase. The Aβ42/Aβ43 forms have a greater tendency to aggregate than Aβ40, which leads to abnormal deposition of Aβ.

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

1 **Antibody-Coated Wells;** 96-well plate
1 vial (6 ml) Human Aβ42 US **Detection Antibody**; contains 0.1% sodium azide, blue dye [1]
1 vial (0.125 ml) **Anti-Rabbit IgG HRP (100X)**
2 vials **Human Aβ42 Standard,** lyophilized; contains 0.1% sodium azide
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**
3 **Adhesive Films**

[1] Colored Standard Diluent Buffer, Detection Antibody, and HRP Diluent give distinctive colors to each step of the ELISA procedure to help prevent pipetting mistakes. The dyes do not interfere with test results.

3. STORAGE INSTUCTIONS – 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)
- AEBSF (4-(2-aminoethyl)-benzenesulfonyl fluoride) or protease inhibitor cocktail containing AEBSF

5. PRECAUCIONS FOR USE

- IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.
- Review the Sample Preparation and Handling in Documents available at biovendor.com
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

6. PREPARATION OF REAGENTS

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.

7. PREPARATION OF SAMPLES

Sample preparation

- Refer to the Sample Preparation and Handling in Documents available at biovendor.com for detailed sample preparation procedures on homogenization of human or transgenic mouse brains.
- 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.
- Analysis of plasma samples may require pretreatment to disrupt interaction of Aβ with masking proteins.

Pre-diluted samples

Because conditions may vary, we recommend that each investigator determine the optimal dilution for each application.

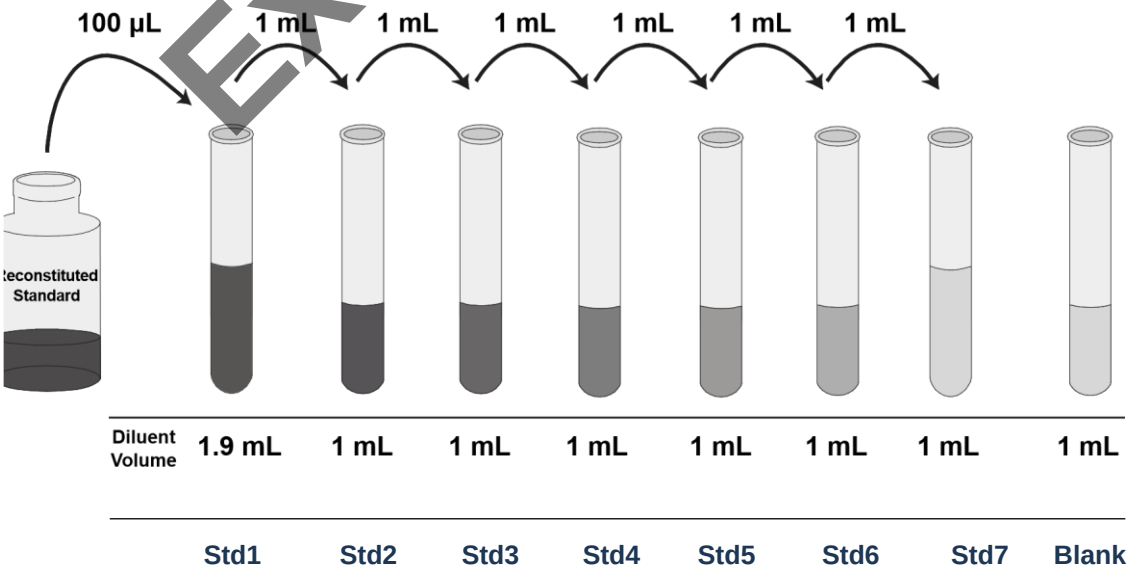
- Dilute samples up to 1:2 fold in Standard Diluent Buffer.
- Dilute samples that are >100 pg/mL with Standard Diluent Buffer.
- Add AEBSF to diluted samples to a final concentration of 1 mM to prevent proteolysis of Aβ peptides.
- Keep samples on ice until ready to apply to plate.

Diluted Standards

Note: Use glass or plastic tubes for diluting standards.

Note: Standards must be diluted using the same composition of buffers used for the diluted experimental samples.

1. Reconstitute Hu Aβ42 US Standard to 2 ng/mL with distilled or deionized water. 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 2 ng/mL human Aβ42. Use the standard within 10 minutes of reconstitution.
2. Add 1.9 mL Standard Diluent Buffer to 1 tube labeled as follows: 100 pg/mL human Aβ42.
3. Add 1 mL Standard Diluent Buffer to each of 7 tubes labeled as follows: 50, 25, 12.5, 6.25, 3.13, 1.56, and 0 pg/mL human Aβ42.
4. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
5. Add AEBSF to diluted standards to a final concentration of 1 mM to prevent proteolysis of Aβ peptides.
6. Return the Standard Diluent Buffer to the refrigerator. Remaining reconstituted standard should be discarded or frozen in aliquots at –80°C for up to 4 months for further use. Standard can be frozen and thawed one time only without loss of immunoreactivity.



2 ng/mL 100 pg/mL 50 pg/mL 25 pg/mL 12.5 pg/mL 6.25 pg/mL 3.13 pg/mL 1.56 pg/mL 0 pg/mL Prepare 1X Anti-Rabbit IgG

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.

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 and add detetector

a) Add 50 µL of standards, controls, or samples (see “Pre-dilute samples”) to the appropriate wells. Leave the wells for chromogen blanks empty
b) Add 50 µL of Human Aβ42 US Detection Antibody solution into each well except the chromogen blanks.
c) Cover the plate with a plate cover and incubate 3 hours at room temperature or overnight at 4°C.
d) Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

Add IgG-HRP

a) Add 100X µL secondary antibody solution 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. PERFORAMNCE CHARACTERISTICS

Standard curve (example)

The following data were obtained for the various standards over the range of 0 to 100 pg/mL human Aβ42.

Standard Human Aβ42 Ultrasensitive (pg/mL)	Optical Density (450 nm)
100	2.53
50	1.39
25	0.81
12.5	0.49
6.25	0.30
3.13	0.22
1.56	0.19
0	0.12

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)	71.30	40.16	21.29
Standard Deviation	5.24	3.96	1.13
% Coefficient of Variation	7.36	9.85	5.32

Intra-assay precision

Samples of known human Aβ42 concentration were assayed in replicates of 16 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	71.76	40.45	21.37
Standard Deviation	5.76	3.75	1.78
% Coefficient of Variation	8.04	9.37	8.33

Linearity of dilution

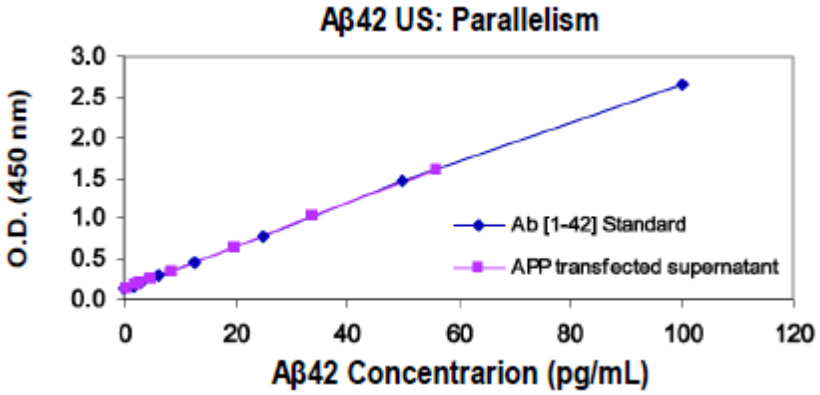
Human CSF containing 280 pg/mL of measured human Aβ42 was diluted 1:2, then serially diluted in Standard Diluent Buffer over the range of the assay. RPMI containing 10% fetal bovine serum was spiked with natural human Aβ42 from APP transfected cells to a level

of 540 pg/mL, initially diluted 1:5, then serially diluted in Standard Diluent Buffer over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 1.0.

Dilution	CSF			Cell Culture Supernatant		
	Measured (pg/mL)	Expected		Measured (pg/mL)	Expected	
		(pg/mL)	%		(pg/mL)	%
1/2	68.99	68.99	100	54.22	54.22	100
1/4	36.90	34.50	107	30.65	27.11	113
1/8	19.43	17.25	113	16.45	13.56	121
1/16	9.09	8.62	105	7.21	6.78	106

Paralelism

Native human Aβ42 was spiked into Standard Diluent Buffer and measured against the standard used in this kit. Parallelism between the two peptides is demonstrated in the following figure.



Recovery

The recovery of native human Aβ42 added to CSF and tissue culture medium containing 10% fetal bovine serum or 10% tissue homogenate was measured on the Human Amyloid beta 42 Ultrasensitive ELISA Kit.

Sample	Average % Recovery
CSF	106.0
RPMI+10% fetal bovine serum	111.0
Tissue homogenate	106.0

Sensitivity

The minimum detectable concentration of human Aβ42 is <1 pg/mL.

This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 64 times and calculating the corresponding concentration..

Specificity

Buffered solutions of a panel of substances were assayed with the Human Amyloid beta 42 Ultrasensitive Elisa kit. The following substances were tested and found to have no cross-reactivity: Aβ [1-12] (100 ng/mL), Aβ [1-20] (100 ng/mL), Aβ [12-28] (100 ng/mL), Aβ

[22-35] (100 ng/mL), Aβ [1-40] (10 ng/mL), Aβ [1-43] (1 ng/mL), Aβ[42-1] (100 ng/mL), α-Synuclein (200 ng/mL), APP (250 ng/mL), and Tau (40 ng/mL).

High-dose hook effect

Samples spiked with human Aβ42 peptide up to 25 ng/mL gave responses higher than that obtained for the highest standard point.

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