BioVendor Group

ENG

Product Data Sheet:

Human Amyloid beta (Aggregated) ELISA

Catalogue number:

RIG018R

For research use only!

BioVendor R&D

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1. INTENDED USE

The Human Amyloid beta (Aggregated) kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). This assay is designed to detect and quantify the level of aggregated beta amyloid (Aβ) in human tissue homogenates, ventricular fluid, CSF, tissue culture supernatant, and buffered solution. The assay recognizes both natural and recombinant human aggregated Aβ, and does not cross-react with mouse or rat. Extracellular amyloid plaques are composed primarily of beta amyloid. Associated with the extracellular plaques are activated microglia and astrocytes. An important neurotoxic form of Aβ is an oligomer, composed of approximately 12 subunits, with a composite molecular weight of 54 kDa. This oligomeric form of Aβ (also known as Amyloid Derived Diffusible Ligand or ADDL) can be separated from fibrillar and protofibrillar forms of aggregated beta amyloid by high speed centrifugation or by size exclusion methods.

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

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.

2. REAGENTS PROVIDED

1 **Antibody Coated Wells.** 12 x 8 Well Strips.
1 vial (11 ml) Human Amyloid beta (Aggregated) **Biotin Conjugate**. Contains 0.1% sodium azide; blue dye*;
1 vial (0.125 ml) **Streptavidin-HRP (100X);** Contains 3.3 mM thymol
2 vials **Human Amyloid beta (Aggregated) Standard:** Contains 0.1% sodium azide; refer to Quality Control Sheet for quantity and reconstitution volume.
1 vial (25 ml) **HRP Diluent.** Contains 3.3 mM thymol; yellow dye*;
1 vial (60 ml) **Standard Diluent Buffer.** Contains 0.1% sodium azide; red dye*;
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**

* In order to help our customers avoid any mistakes in pipetting the ELISAs, we provide colored Standard Diluent Buffer, Detection Antibody, and HRP Diluent to help monitor the addition of solutions to the reaction wells. This does not in any way interfere with the test results.

3. STORAGE INSTRUCTIONS

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

4. MATERIALS REQUIRED BUT NOT PROVIDED

- Microtiter plate reader capable of measurement at or near 450 nm.
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents
- Distilled or deionized water.
- Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).

5. PRECAUTIONS FOR USE AND SAFETY

- 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

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 Prepare Tissue Extraction Buffer

Note: See the Sample Preparation and Handling for detailed information on preparing Tissue Extraction Buffer.

1. Prepare 5 mL of Tissue Extraction Buffer. Tissue Extraction Buffer consists of 25 mM Tris (pH 7.4), and 150 mM NaCl.
2. Immediately before use, add 1.46 µM pepstatin A, 0.154 µM aprotinin, 2.03 µM leupeptin, 0.5 mM AEBSF, and 0.29 mM PMSF.

If desired, supplement the buffer with phosphatase inhibitors: 0.05 mM fenvalerate, 0.05 mM cantharidin, 1 mM Na3VO4, 1 mM Na4P2O7, and 50 mM NaF.

7. PREPARATION OF SAMPLES

7.1. Prepare aggregated Aβ sample (liquid)

Due to shared epitopes, the following analytes may cause interference with this assay: APP, monomeric Aβ40, monomeric Aβ42, protofibrillar Aβ, and fibrillar Aβ. Sample preparation should therefore be carefully considered when using this assay.

1. Collect samples in pyrogen/endotoxin-free polypropylene tubes.
   Note: Aggregated Aβ is sensitive to freeze-thaw cycles. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
2. Clarify samples by centrifugation prior to analysis.
   - Centrifuge at 14,000 × g for 10 minutes to minimize fibrils in aggregated Aβ-containing samples.
   - Centrifuge at 100,000 × g for 1 hour at 4°C to minimize fibrils and protofibrils.

Size exclusion methods, such as gel permeation chromatography or ultrafiltration, can also improve assay performance.

7.2. Prepare tissue homogenate

Prepare tissue homogenate

1. Determine the mass of the tissue sample in a microcentrifuge tube.
2. Add 10 × the tissue mass of Tissue Extraction Buffer, then homogenize thoroughly.
3. Sonicate the homogenized tissue sample (2 blasts, 10 seconds each).
4. Centrifuge the homogenate at 100,000 × g for 1 hour at 4°C.
5. Carefully transfer the clear supernatant fraction into clean microcentrifuge tubes on ice.

The supernatant fraction contains TBS-soluble aggregates of Aβ.

7.3. Pre-dilute samples

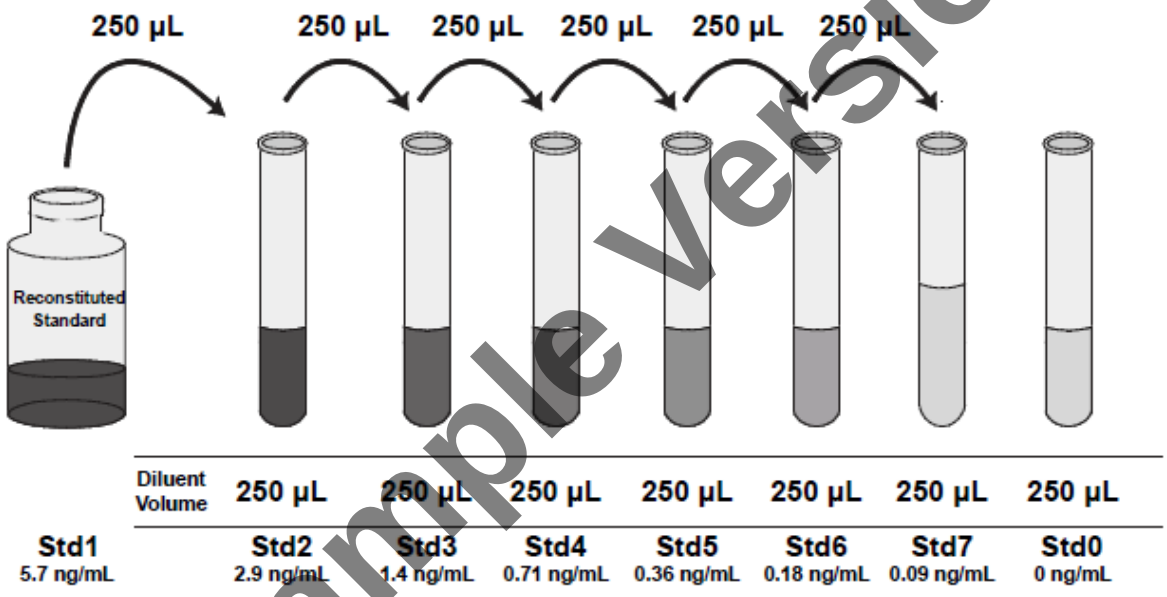
Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

- Perform sample dilutions with Standard Diluent Buffer.
- Dilute samples prepared in Tissue Extraction Buffer 1:2 or greater in Standard Diluent Buffer (e.g., 25 µL sample into 25 µL buffer).

7.4. Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Reconstitute Hu Aggregated Aβ Standard to 5.7 ng/mL with Standard Diluent Buffer. 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 5.7 ng/mL human aggregated Aβ. Use the standard within 1 hour of reconstitution.
2. Add 250 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 2.9, 1.4, 0.71, 0.36, 0.18, 0.09, and 0 ng/mL human aggregated Aβ.
3. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
4. 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.



7.5. Prepare 1X Streptavidin-HRP solution

Note: Prepare 1X Streptavidin-HRP within 15 minutes of usage.

The Streptavidin-HRP (100X) is in 50% glycerol, which is viscous. To ensure accurate dilution:

1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, wipe the pipette tip with clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of Streptavidin-HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-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.
- Total assay time: 4 hours

Bind antigen

a. Add 100 µL of standards, controls, or to the appropriate wells. Leave the wells for chromogen blanks empty.

b. Cover the plate with a plate cover and incubate for 2 hours at room temperature.

c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

Add Biotin Conjugate

a. Add 100 µL Hu Aggregated Aβ Biotin Conjugate solution into each well except the chromogen blanks.

b. Cover the plate with plate cover and incubate for 1 hour at room temperature.

c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

Add Streptavidin-HRP

a. Add 100 µL 1X Streptavidin-HRP solution into each well except the chromogen blanks.

b. Cover the plate with a plate cover and incubate for 30 minutes at room temperature.

c. Thoroughly aspirate the solution from the wells 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

The following data were obtained for the various standards over the range of 0 to 5.7 ng/mL Human aggregated Aβ.

Standard Human Amyloid beta (Aggregated) ng/mL	Optical Density (450 nm)
5.7	3.09
2.9	2.22
1.4	1.46
0.71	0.57
0.36	0.37
0.18	0.26
0.09	0.17
0	0.09

Sensitivity

The analytical sensitivity of this assay is <0.01 ng/mL of aggregated Aβ. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.

Inter-assay precision

Samples were assayed 48 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	2.82	1.36	0.33
Standard Deviation	0.16	0.12	0.03
% Coefficient of Variation	5.74	8.99	7.61

Intra-assay precision

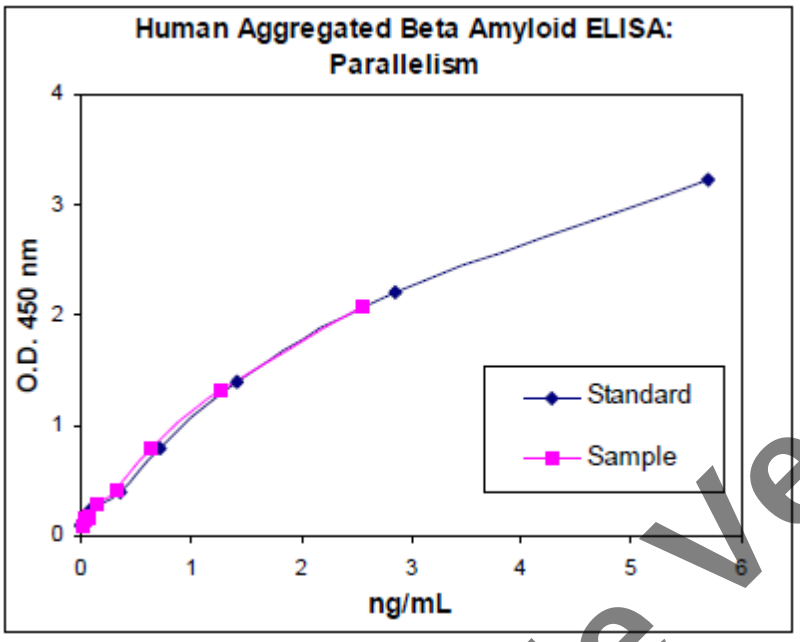
Samples of known aggregated Aβ concentrations were assayed in replicates of 16 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	2.84	1.48	0.32
Standard Deviation	0.19	0.09	0.03
% Coefficient of Variation	6.68	6.29	9.26

Parallelism

Aggregated Aβ peptide was serially diluted in Standard Diluent Buffer.

The optical density of each dilution was plotted against the aggregated Aβ standard curve. Parallelism demonstrated by the figure below indicates that the standard accurately reflects aggregated Aβ content in a sample.



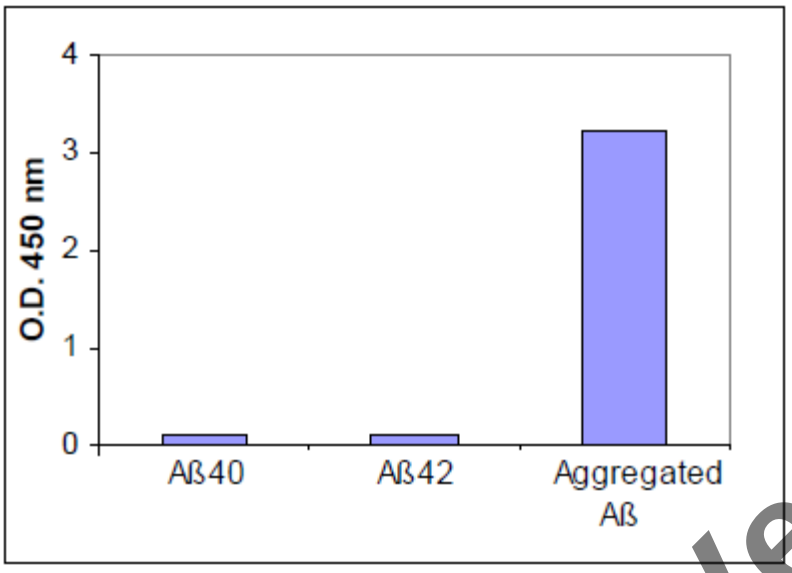
Linearity of dilution

Aggregated Aβ was diluted in Standard Diluent Buffer over the range of the assay and measured. Linear regression analysis of sample values versus the expected concentrations yielded a correlation coefficient of 0.99.

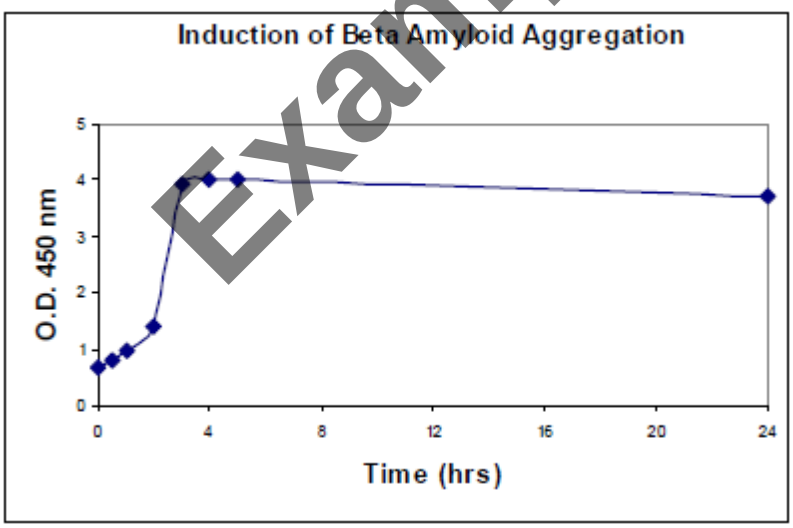
Dilution	Measured (pg/mL)	Expected	
		Conc. (pg/mL)	%
Neat	2.55	2.55	100
1/2	1.35	1.28	106
1/4	0.737	0.638	115
1/8	0.344	0.319	108
1/16	0.214	0.159	134
1/32	0.088	0.079	110

Specificity

The specificity of the Human Aggregated Beta Amyloid ELISA Kit for human aggregated Aβ was determined by measuring samples containing Aβ40 peptide, Aβ42 peptide, and aggregated Aβ. The data confirms that the assay recognizes the aggregated form of Aβ, but not the nonaggregated Aβ40 or Aβ42 peptides.



Human Aβ40 peptides was treated with 100% HFIP at 1 mg/mL for 1 hour then dried over a gentle stream of nitrogen gas. When all of the HFIP solution was evaporated, the peptide was resuspended with water and incubated at 37°C. Samples were collected over a 24 hour period and analyzed in the Human Aggregated Beta Amyloid ELISA. The data demonstrate that Aβ aggregation appeared to reach maximal level at 4 hours post-reconstitution.



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