



Enabling Legendary Discovery™ Human Amyloid-Beta (1-42)

ELISA MAX™ Deluxe Set

Cat. No. 448704



BioLegend's ELISA MAX™ Deluxe Set contains the components necessary for the accurate quantification of natural and recombinant human Amyloid-Beta (1-42). The set is designed for cost-effective and accurate quantification of human Amyloid-Beta (1-42) in serum, plasma and cerebrospinal fluid samples. BioLegend's ELISA MAX™ Deluxe Sets are sensitive, accurate, and robust.

It is highly recommended that this instruction sheet be read in its entirety before using this product. Do not use this set beyond the expiration date.

Materials Provided

1. Human Amyloid-Beta (1-42) Capture Antibody (200X)
2. Human Amyloid-Beta (1-42) Detection Antibody (200X)
3. Human Amyloid-Beta (1-42) Lyophilized Standard
4. Streptavidin-Polymer HRP (1000X)
5. Substrate Solution D
6. Coating Buffer B (5X)
7. ELISA Diluent 1 (20X)
8. Blocking Buffer A (2X)

Introduction

The Amyloid peptides are peptides of 36-43 amino acids which are produced through the post-translational cleavage of the Amyloid Pre-cursor Protein by beta and gamma secretase. Although numerous different Amyloid-Beta species exist, the majority of the Amyloid-Beta peptides produced by post-translational modification consists of either Amyloid-Beta (1-40) (80-90%) and Amyloid-Beta (1-42) (5-10%). Both variants are major components of the extracellular plaques found in Alzheimer's brain. However, Amyloid-Beta (1-42) was reported to be more prone to aggregation than the Amyloid-Beta (1-40) variant despite differing in only 2 amino acids at the C terminus. Other studies that aimed at characterizing the difference in aggregation tendency between the two variants suggested that they have distinct 3D structures in solution.

Principle of the Test

BioLegend ELISA MAX™ Deluxe Set contains pre-optimized essential reagents and additional buffers and solutions for Sandwich ELISA assay. A human Amyloid-Beta (1-42) specific capture antibody is first coated on a 96-well plate. Standards and samples are added to the wells, and human Amyloid-Beta (1-42) binds to the immobilized capture antibody. Next, a biotinylated anti-human Amyloid-Beta (1-42) detection antibody is added, producing an antibody-antigen-antibody "sandwich". The Streptavidin-Polymer HRP reagent is subsequently added, followed by TMB Substrate, producing a blue color in proportion to the concentration of human Amyloid-Beta (1-42) present in wells. Then stop solution should be added to wells to terminate the reaction. This step changes the reaction color from blue to yellow. The absorbance in wells should be read at 450nm and 570nm using a microplate reader.

For research purposes only. Not for use in diagnostic or therapeutic procedures.

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Materials to be Provided by the End-User

- Microwell plates: BioLegend Cat. No. 423501 is recommended
- Wash Buffer: BioLegend Cat. No. 421601 is recommended, or PBS + 0.05% Tween-20
- Stop Solution: BioLegend Cat. No. 423001 is recommended, or acid solution, e.g. 2N H2SO4
- Plate Sealers: BioLegend Cat. No. 423601 is recommended
- Deionized (DI) water
- A microplate reader capable of measuring absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 2 µL to 1 mL
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer

Storage Information

- Store kit components between 2°C and 8°C.
- After reconstitution of the lyophilized standard with 1X ELISA Diluent 1, aliquot into polypropylene vials and store at -70°C for up to one month. Avoid repeated freeze/thaw cycles.
- Prior to use, bring all components to room temperature (18°C-25°C). Upon assay completion return all components to appropriate storage conditions.

Health Hazard Warnings

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details (<http://www.biolegend.com/msds>).
2. Substrate Solution D is harmful if ingested. Additionally, avoid skin, eye or clothing contact.
3. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling

Cerebrospinal Fluid: If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at < -70°C. Avoid repeated freeze/thaw cycles.

Serum: Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at 1,000 X g. Remove serum layer and assay immediately or store serum samples at < -20°C. Avoid repeated freeze/thaw cycles. Serum specimens should be clear and non-hemolyzed.

Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1,000 X g within 30 minutes of collection. Assay immediately or store plasma samples at < -20°C. Avoid repeated freeze/thaw cycles. Plasma specimens should be clear and non-hemolyzed.

Reagent and Sample Preparation

Do not mix reagents from different sets or lots. Reagents and/or antibodies from different manufacturers should not be used with this set. All reagents should be diluted immediately prior to use.

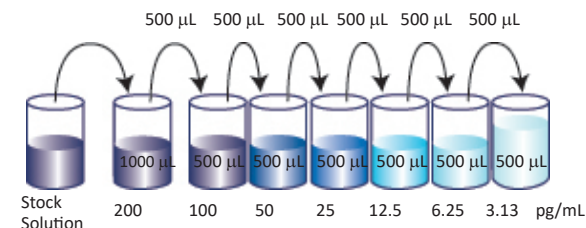
NOTE: Precipitation of ELISA Diluent 1 (20X) may be observed when stored long term between 2°C and 8°C. The precipitation does not alter the performance of the Buffer. If heavy precipitation is observed after the dilution to 1X ELISA Diluent 1, it can be filtered to clarify the solution.

Preparation of 1X Reagent for 1 Plate

Material	Dilute with
2.4 mL of Coating Buffer B (5X)	9.6 mL of Deionized Water
60 µL of Capture Antibody (200X)	12 mL of 1X Coating Buffer B
12 mL Blocking Buffer A (2X)	12 mL of Deionized Water
2.5 mL ELISA Diluent 1 (20X)	47.5 mL of Deionized Water
60 µL of Detection Antibody (200X)	12 mL of 1X ELISA Diluent 1
12 µL of Streptavidin-Polymer HRP (1000X)	12 mL of 1X ELISA Diluent 1 (1000X)

Lyophilized vials are under vacuum pressure. Reconstitute the Human Amyloid-Beta (1-42) Standard by adding the volume of 1X ELISA Diluent 1 to make the 22.1 ng/mL standard stock solution (Refer to ELISA MAX Kit Lot-Specific Certificate of Analysis/ELISA MAX Kit Protocol). Allow the reconstituted standard to sit for 15 minutes at room temperature, then mix gently prior to making dilutions.

Prior to use, prepare 1,000 µL of the top standard at a concentration of 200 pg/mL from the stock solution in 1X ELISA Diluent 1. Perform six two-fold serial dilutions of the 200 pg/mL top standard with 1X ELISA Diluent 1 in separate tube. Mix gently to avoid foaming of diluent. After diluting, the Human Amyloid-Beta (1-42) standard concentrations are 200 pg/mL, 100 pg/mL, 50 pg/mL, 25 pg/mL, 12.5 pg/mL, 6.25 pg/mL, and 3.13 pg/mL, respectively. 1X ELISA Diluent 1 serves as the zero standard (0 pg/mL).



Samples: In general, a 32-folds dilution in 1X ELISA Diluent 1 is recommended for cerebrospinal fluid samples. No dilution is needed for serum and plasma samples. Samples can be diluted further in 1X ELISA Diluent 1 to fit within the range of the assay as determined by the end user.

Assay Procedure

Do not use sodium azide in any solutions as it inhibits the activity of the horseradish-peroxidase enzyme.

1. One day prior to running the ELISA, dilute Capture Antibody in 1X Coating Buffer B as described in Reagent Preparation. Add 100 µL of this Capture Antibody solution to all wells of a 96-well plate. Seal plate and incubate overnight (16-18 hrs) between 2°C and 8°C.
2. Bring all reagents to room temperature (RT) prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay
3. Wash plate 4 times with at least 300 µL Wash Buffer per well and blot residual buffer by firmly tapping plate upside down on absorbent paper. **All subsequent washes should be performed similarly.**
4. To block non-specific binding and reduce background, add 200 µL 1X Blocking Buffer A per well.
5. Seal plate and incubate at RT for 1 hour with shaking on a plate shaker (e.g. 500 rpm with a 0.3 cm circular orbit). All subsequent incubation with shaking should be performed similarly.

6. While plate is being blocked, prepare standards and appropriate sample dilutions if necessary.
7. Wash plate 4 times with Wash Buffer.
8. Add 100 μ L of standards and samples to the appropriate wells. If dilution is required, samples should be diluted in 1X ELISA Diluent 1 before adding to the wells.
9. Seal plate and incubate at RT for 2 hours with shaking.
10. Wash plate 4 times with Wash Buffer.
11. Add 100 μ L of diluted Detection Antibody solution to each well, seal plate and incubate at RT for 1 hour with shaking.
12. Wash plate 4 times with Wash Buffer.
13. Add 100 μ L of diluted Streptavidin-Polymer HRP solution to each well.
14. Seal plate and incubate at RT for 30 minutes with shaking.
15. Wash plate 5 times with Wash Buffer. For this final wash, soak wells in Wash Buffer for 1 minute for each wash. This will help minimize background.
16. Add 100 μ L of TMB Substrate Solution and incubate **in the dark** for 10 minutes*. Positive wells should turn blue in color. It is not necessary to seal the plate during this step.
17. Stop reaction by adding 100 μ L of Stop Solution to each well. Positive wells should turn from blue to yellow.
18. Read absorbance at 450 nm and 570 nm within 15 minutes, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

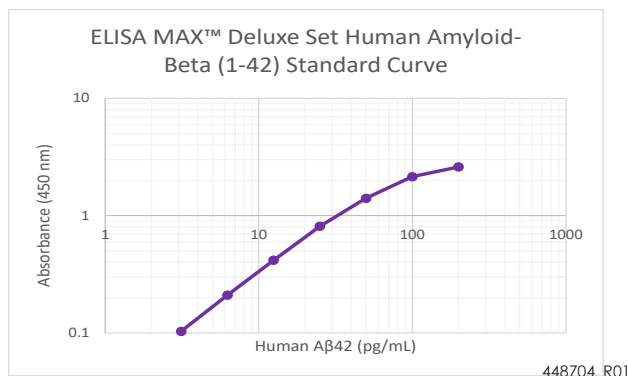
*Optimal substrate incubation time depends on laboratory conditions and the optical linear ranges of ELISA plate readers

Calculation of Results

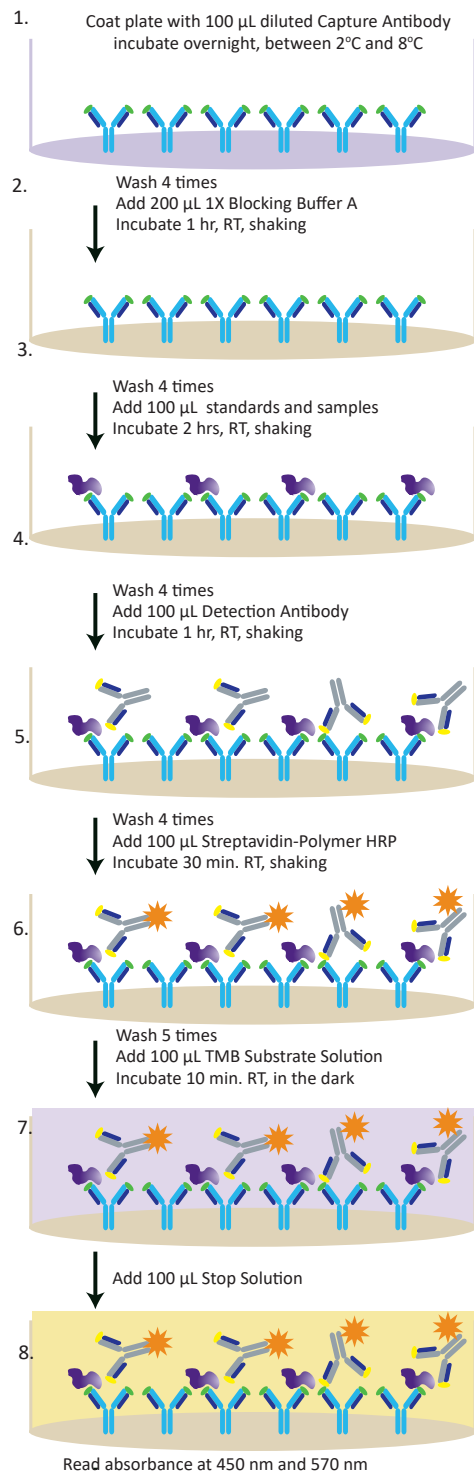
Plot the standard curve on log-log axis graph paper with analyte concentration on the x-axis and absorbance on the y-axis. Draw a best fit line through the standard points. To determine the unknown analyte concentrations in the samples, find the absorbance value of the unknown on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the corresponding analyte concentration. If the samples were diluted, multiply by the appropriate dilution factor. The data is best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If a test sample's absorbance value falls outside the standard curve ranges, that test sample needs to be reanalyzed at a higher or lower dilution as appropriate.

Typical Data

Standard Curve: This standard curve was generated at BioLegend for demonstration purposes only. A standard curve must be run with each assay.



Assay Procedure Summary



Performance Characteristics

Sensitivity: The minimum detectable concentration of Human Amyloid Beta (1-42) is 0.718 \pm 0.190 pg/mL.

Specificity: This kit recognizes natural and synthetic Human Amyloid Beta (1-42). No significant cross reactivity was observed when this kit was used to analyze the following synthetic human proteins at 10,000 pg/mL: Amyloid-Beta (1-38) (0%), Amyloid-Beta (1-40) (0%), and Amyloid-Beta (1-43) (1.37%).

Troubleshooting

High Background:

- Background wells were contaminated.
- Matrix used had endogenous analyte.
- Plate was insufficiently washed.
- TMB Substrate Solution was contaminated.
- Streptavidin-Polymer HRP had prolonged exposure to light.

No signal:

- Incorrect or no antibodies were added.
- Streptavidin-Polymer HRP was not added.
- Substrate solution was not added.
- Wash buffer contains sodium azide.

Low or poor signal for the standard curve:

- Standard was incompletely reconstituted or was stored improperly.
- Reagents were added to wells with incorrect concentrations.
- Plate was incubated with improper temperature, timing or agitation.

Signal too high, standard curves saturated:

- Standard was reconstituted with less volume than required.
- One or more reagent incubation steps were too long.
- Plate was incubated with inappropriate temperature, timing, or agitation.

Sample readings out of range:

- Samples contain no or below detectable levels of analyte.
- Samples contain analyte concentrations greater than highest standard point.

High variations in samples and/or standards:

- Pipetting errors may have occurred.
- Plate washing was inadequate or nonuniform.
- Samples were not homogenous.
- Samples or standard wells were contaminated.

BioLegend, Inc.

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 email: techserv@biolegend.com