BioLegend®

LEGEND MAX™

ELISA Kit with Pre-coated Plates

Human Amyloid beta (1-40)

Cat. No. 449007

ELISA Kit for Accurate Quantitation of Human Amyloid beta (1-40) in Cerebrospinal Fluid, Serum, Plasma, and Other Biological Fluids

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

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Introduction:

Human amyloid beta (1-40) is a peptide fragment implicated in the onset and progression of Alzheimer's disease, due to its presence in amyloid plaques that are a hallmark of the disease. This peptide, along with amyloid beta (1-42) and other amyloid isoforms, is generated from the cleavage of amyloid precursor protein (APP). Amyloid beta (1-40) and amyloid beta (1-42) are the two most common isoforms in the brain. While amyloid beta (1-40) is more prevalent, amyloid beta (1-42) is the major component of amyloid plaques. Nevertheless, amyloid beta (1-40) is relevant for Alzheimer's research because the amyloid beta (1-42)/amyloid beta (1-40) ratio is an important diagnostic for the severity of Alzheimer's disease.

The BioLegend LEGEND MAX™ Human Amyloid beta (1-40) ELISA Kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with a mouse monoclonal anti-human amyloid beta (1-40) antibody. The detection antibody is a biotinylated mouse monoclonal anti-human amyloid beta (1-40) antibody. This kit is specifically designed for the accurate quantitation of human amyloid beta (1-40) in cerebrospinal fluid, serum, plasma, and other biological fluids. This kit is analytically validated with ready-to-use reagents.

Materials Provided:

Description	Quantity	Volume (per bottle)	Part #
Anti-human Amyloid beta (1-40) Pre-coated 96-well Strip Microplate	1 plate		750002740
Human Amyloid beta (1-40) Detection Antibody (200X)	1 bottle	0.3 mL	750004310
Human Amyloid beta (1-40) Lyophilized Standard	1 vial	lyophilized	750002745
Streptavidin-PolyHRP	1 bottle	12 mL	750002747
Assay Buffer E	1 bottle	25 mL	79559
Assay Buffer B	1 bottle	25 mL	79128
Wash Buffer (20X)	1 bottle	50 mL	78233
Substrate Solution D	1 bottle	12 mL	78115
Stop Solution	1 bottle	12 mL	79133
Plate Sealers	1 pack		78101

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Materials to be Provided by the End-User:

- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 µL to 1,000 µL
- Deionized water
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer
- Polypropylene vials

Storage Information:

Store unopened kit components between 2°C and 8°C. Do not use this kit beyond its expiration date.

<table>
<tr><th colspan="2">Opened or Reconstituted Components</th></tr>
<tr><td>Microplate wells</td><td>If not all microplate strips are used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal. Store between 2°C and 8°C for up to one month.</td></tr>
<tr><td>Standard</td><td>The remaining reconstituted standard stock solution can be aliquoted into polypropylene vials and stored at -70°C for up to one month. Avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Detection Antibody (200X)</td><td rowspan="6">Store opened reagents between 2°C and 8°C and use within one month.</td></tr>
<tr><td>Streptavidin-PolyHRP</td></tr>
<tr><td>Assay Buffer E</td></tr>
<tr><td>Wash Buffer (20X)</td></tr>
<tr><td>Substrate Solution D</td></tr>
<tr><td>Stop Solution</td></tr>
</table>

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online at BioLegend's website for details (www.biolegend.com/msds).
2. Substrate Solution D is harmful if inhaled or ingested. Avoid skin, eye, and clothing contact.

3. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma, and other biological fluids in accordance with NCCLS regulations.
4. Stop Solution contains strong acid. *Wear eye, hand, and face protection.*
5. Before disposing of the plate, rinse it with an excess amount of tap water.

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. If possible, unknown samples should be run at a number of dilutions to determine the optimal dilution factor that will ensure accurate quantitation.

Cell Culture Supernatant: If necessary, centrifuge all samples to remove debris prior to analysis. It is recommended that samples 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 < -70°C. Avoid repeated freeze-thaw cycles.

Plasma: Collect blood samples in citrate, heparin, or EDTA containing tubes. Centrifuge for 10 minutes at 1,000 x *g* within 30 minutes of collection. Assay immediately or store plasma samples at < -70°C. Avoid repeated freeze-thaw cycles.

Reagent and Sample Preparation:

Note: All reagents should be diluted immediately prior to use.

1. Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water. If crystals have formed in the 20X Wash Buffer, bring to room temperature and vortex until dissolved.
2. Reconstitute the Human Amyloid beta (1-40) Lyophilized Standard by adding the volume of Assay Buffer E to make the 20 ng/mL standard stock solution (refer to LEGEND MAX Kit Lot-Specific Certificate of Analysis/ LEGEND MAX Kit Protocol). Allow the reconstituted standard to sit at room temperature for 15-20 minutes, then briefly vortex to mix completely.
3. Dilute the 200X Human Amyloid beta (1-40) Detection Antibody to 1X with Assay Buffer B. For example, make 12 mL of 1X Detection Antibody by adding 60 µL of 200X Detection Antibody to 11.94 mL of Assay Buffer B.

4. Dilute cerebrospinal fluid (CSF) samples 1:20 in Assay Buffer E before use. In general, serum and plasma samples are analyzed without dilutions. However, if dilutions are required, use Assay Buffer E as the sample diluent.

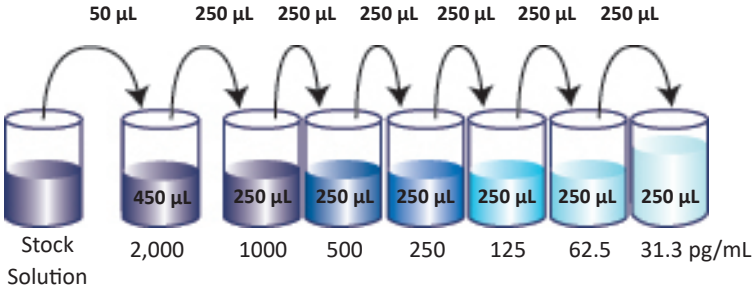
Assay Procedure:

Note: Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this kit. **A plate shaker is not necessary for this assay.**

1. Bring all reagents to room temperature 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.

2. If not all microplate strips will be used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.

3. Prepare 500 µL of the 2,000 pg/mL top standard by diluting 50 µL of the standard stock solution in 450 µL of Assay Buffer E. Perform six two-fold serial dilutions of the 2,000 pg/mL top standard in separate tubes using Assay Buffer E as the diluent. Thus, the human amyloid beta (1-40) standard concentrations in the tubes are 2,000 pg/mL, 1000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL and 31.3 pg/mL, respectively. Assay Buffer E serves as the zero standard (0 pg/mL).



4. Wash the plate 4 times with at least 300 µL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping the plate upside down on absorbent paper. All subsequent washes should be performed similarly.

5. Add 50 µL of Assay Buffer E to each well that will contain either standard dilutions or samples.

6. Add 50 µL of standard dilutions or samples to the appropriate wells.

7. Seal the plate with a plate sealer included in the kit and incubate the plate at **4°C** for 2 hours.

8. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
9. Add 100 µL of 1X Human Amyloid beta (1-40) Detection Antibody to each well, seal the plate and incubate at **4°C** for 1 hour.
10. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
11. Add 100 µL of Streptavidin-PolyHRP to each well, seal the plate and incubate at room temperature for 30 minutes **on the benchtop**.
12. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 4. For this final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
13. Add 100 µL of Substrate Solution D to each well and incubate at room temperature for 10 minutes **in the dark**.* Wells containing human amyloid beta (1-40) should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.
14. Stop the reaction by adding 100 µL of Stop Solution to each well. The solution color should change from blue to yellow.
15. Read absorbance at 450 nm within 15 minutes. If the reader is capable of reading at 570 nm, 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.**

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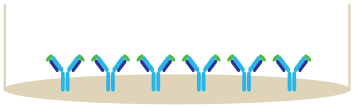
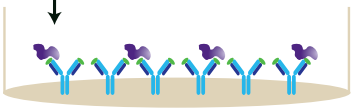
Assay Procedure Summary

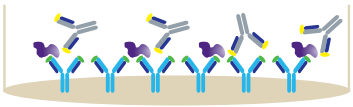
1. Wash 4 times
Add 50 µL Assay Buffer E



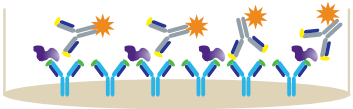
2. Add 50 µL diluted standards or samples.
Incubate 2 hr, **4°C**.



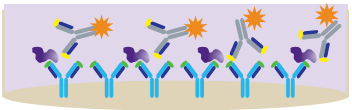
3. Wash 4 times
Add 100 µL 1X Detection Antibody.
Incubate 1 hr, **4°C**.



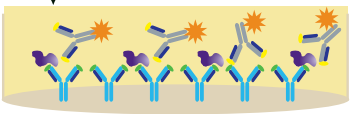
4. Wash 4 times
Add 100 µL Streptavidin-PolyHRP.
Incubate 30 min, **RT**, **on benchtop**.



5. Wash 5 times.
Add 100 µL Substrate Solution D.
Incubate 10 min*, **RT**, **in the dark**.



6. Add 100 µL Stop Solution.



7. Read absorbance at 450 nm and 570 nm.

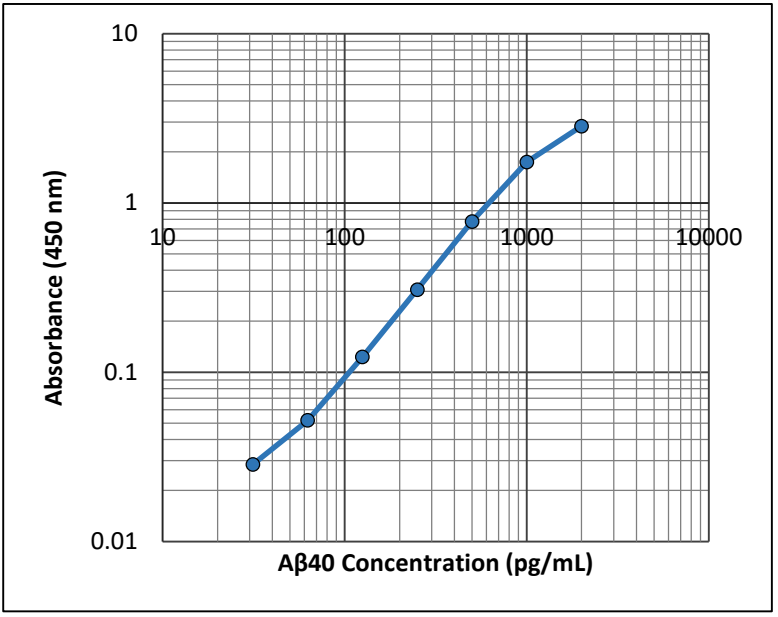
Calculation of Results:

The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If the appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate standards, controls, and samples. Plot the standard curve on log-log graph paper with amyloid beta (1-40) concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown amyloid beta (1-40) concentrations, find the mean absorbance value of the unknown concentration 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 concentration.

If samples were diluted, multiply the concentration by the appropriate dilution factor. If a test sample's absorbance value falls outside the linear portion of the standard curve, the test sample needs to be re-analyzed at a higher (or lower) dilution, as appropriate.

Typical Data:

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



Performance Characteristics:

Specificity: No cross reactivity was observed when this kit was used to analyze APP770, amyloid beta (1-38), and amyloid beta (1-43) at up to 50 ng/mL. At 50 ng/mL, there was less than 1.1% cross-reactivity with amyloid beta (1-42).

Sensitivity: The minimum detectable concentration of amyloid beta (1-40) is 20.4 pg/mL.

Recovery: Recombinant human amyloid beta (1-40) was spiked into CSF, serum, plasma, and cell medium at concentrations of 1000 pg/mL, 250 pg/mL, and 62.5 pg/mL then analyzed with the LEGEND MAX™ Human Amyloid beta (1-40) ELISA kit.

Sample Type	N	Recovery (%)
Cerebrospinal Fluid	3	106.3
Serum	3	82.2
Citrate Plasma	3	83.7
EDTA Plasma	3	82.6
Heparin Plasma	3	89.5
Cell Culture Medium	1	83.5

Linearity: Four human CSF samples were serially diluted to produce samples with concentrations within the dynamic range. Human serum and plasma samples were spiked with a high concentration of amyloid beta (1-40) then serially diluted.

Sample Type	N	Linearity (%)
Cerebrospinal Fluid	4	85.4
Serum	4	109.1
Citrate Plasma	3	113.0
EDTA Plasma	3	109.5
Heparin Plasma	3	96.0
Cell Culture Medium	1	110.8

Intra-Assay Precision: Sixteen replicates of each of two samples containing different human amyloid beta (1-40) concentrations were tested in one assay.

Concentration	Sample 1	Sample 2
Number of Replicates	16	16
Mean Concentration (pg/mL)	573.9	46.0
Standard Deviation	31.8	4.0
%CV	5.5	8.7

Inter-Assay Precision: Two samples containing different concentrations of human amyloid beta (1-40) were tested in ten independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	10	10
Mean Concentration (pg/mL)	578.9	48.9
Standard Deviation	41.4	6.5
%CV	7.2	13.3

Biological Samples: Human CSF samples, including samples from Alzheimer's disease patients, were tested using the LEGEND MAX™ Human Amyloid beta (1-40) ELISA kit. Also, human serum and plasma (citrate, EDTA, and heparin) samples were tested.

	CSF	Serum	Citrate Plasma	EDTA Plasma	Heparin Plasma
N	4	3	3	3	3
Min (pg/mL)	2848.0	144.8	138.3	193.0	182.9
Max (pg/mL)	7658.4	431.6	181.3	217.2	271.3
Mean (pg/mL)	4624.7	288.3	162.9	202.1	225.1

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Troubleshooting Guide:

Problem	Probable Cause	Solution
High Background	Background wells were contaminated	Avoid cross-well contamination by using the provided plate sealers. Use multichannel pipettes and change tips between pipetting samples and reagents.
	Insufficient washes	Increase number of washes. Increase soaking time between washes prior to addition of substrate solution.
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells.
No or poor signal	Detection Antibody, Avidin-HRP or Substrate solution were NOT added	Rerun the assay and follow the protocol.
	Wrong reagent or reagents were added in wrong sequential order	
	Insufficient plate agitation	The plate should be agitated during all incubation steps using a plate shaker at a speed where solutions in wells are within constant motion without splashing.
	The wash buffer contains Sodium Azide (NaN3)	Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity.
	Incubations were done at an inappropriate temperature, timing or without agitation	Rerun the assay and follow the protocol.
Low or poor standard curve signal	The standard was incorrectly reconstituted or diluted	Adjust the calculations and follow the protocol.
	Standard was inappropriately stored	Store the reconstituted standard stock solution in polypropylene vials at -70°C. Avoid repeated freeze-thaw cycles.
	Reagents added to wells with incorrect concentrations	Check for pipetting errors and the correct reagent volume.

Problem	Probable Cause	Solution
Signal is high, standard curves have saturated signal	Standard reconstituted with less volume than required	Reconstitute new lyophilized standard with the correct volume of solution recommended in the protocol.
	Standards/samples, detection antibody, Avidin-HRP or substrate solution were incubated for too long	Rerun the assay and follow the protocol.
Sample readings are out of range	Samples contain no or below detectable levels of the analyte	If samples are below detectable levels, it may be possible to use a larger sample volume. Contact technical support for appropriate protocol modifications.
	Samples contain analyte concentrations greater than highest standard point	Samples may require dilution and analysis.
High variation in samples and/or standards	Multichannel pipette errors	Confirm that pipette calibrations are accurate.
	Plate washing was not adequate or uniform	Ensure pipette tips are tightly secured. Ensure uniformity in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before assaying.
	Samples may have high particulate matter	Remove particulate matter by centrifugation.
	Cross-well contamination	Do not reuse plate sealers. Always change tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.

ELISA Plate Template

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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