Human Aß40 ELISA Kit

Catalog Number KHB3481 (96 tests)

Pub. No. MAN0014562 **Rev.** 3.0 (30)

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™ Human Aβ40 ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of human Aβ40 in samples (e.g., tissue culture medium, tissue homogenate, cerebrospinal fluid (CSF), etc.). The assay will recognize both natural and synthetic forms of human Aβ40.

Contents and storage

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

Contents	Cat. No. KHB3481 (96 tests)
Hu Aß40 Standard, lyophilized synthetic peptide; contains 0.1% sodium azide.	1 vial
Standard Diluent Buffer; contains 0.1% sodium azide, red dye [1]	60 mL
Antibody Coated Wells, 96-well plate	1 plate
Hu Aß40 Detection Antibody; contains 0.1% sodium azide, blue dye [1]	6 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 and less toxic than PMSF) at a 1 mM final concentration is very helpful. Keep samples on ice until ready to apply to plate.

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.

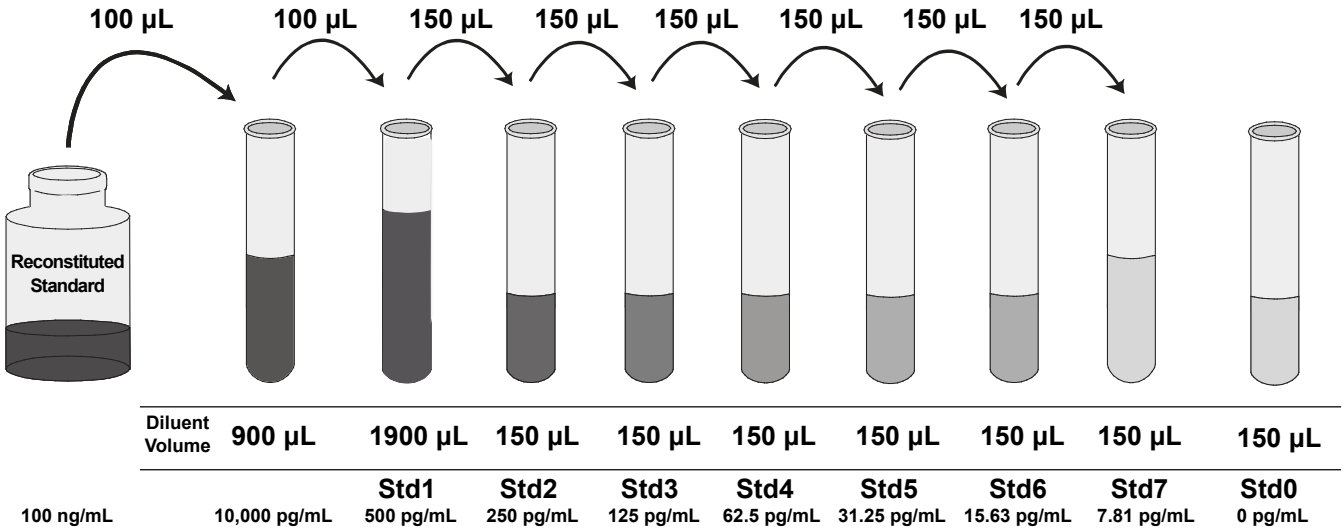
Dilute standards

Note: The Hu Aβ40 Standard is calibrated against highly purified human Aβ40 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).

Note: Use plastic tubes for diluting standards. Do not use glass tubes.

1. Reconstitute Hu Aβ40 Standard to 100 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 100 ng/mL human Aβ40. **Use the standard within 1 hour of reconstitution.**
2. Add 100 µL Reconstituted Standard to one tube containing 900 µL Standard Diluent Buffer and mix. Label as 10,000 pg/mL human Aβ40.
3. Add 100 µL of 10,000 pg/mL standard to one tube containing 1,900 µL Standard Diluent Buffer and label as 500 pg/mL human Aβ40.
4. Add 150 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 250, 125, 62.5, 31.25, 15.63, 7.81, and 0 pg/mL human Aβ40.
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 further use. Standard can be frozen and thawed one time only without loss of immunoreactivity.



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 and add detector**

a. Add 50 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. Add 50 µL of Hu Aβ40 Detection Antibody solution into each well except the chromogen blanks.
c. Tap the side of the plate to mix. Cover the plate with a plate cover and incubate 3 hours at room temperature.
d. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 **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.

3 **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.

4 **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 30 minutes 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 was obtained for the various standards over the range of 0 to 500 pg/mL human Aβ40.

Standard Human Aß40 (pg/mL)	Optical Density (450 nm)
500	3.89
250	2.43
125	0.95
62.5	0.39
31.25	0.19
15.63	0.13
7.81	0.10
0	0.08

Inter-assay precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	50.8	130.0	298.2
Standard Deviation	2.0	4.5	12.1
% Coefficient of Variation	3.9	3.5	4.1

Intra-assay precision

Samples of known human Aβ40 concentration were assayed in replicates of 14 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	49.5	129.7	305.7
Standard Deviation	1.4	3.3	7.1
% Coefficient of Variation	2.8	2.5	2.3

Cross-reactivity

Rodent β Amyloid (1-40) showed approximately 0.5% cross reaction at 50 ng/mL.

High-dose hook effect

Samples spiked with human Aβ40 peptide up to 1 µg/mL gave responses higher than that obtained for the last standard point.

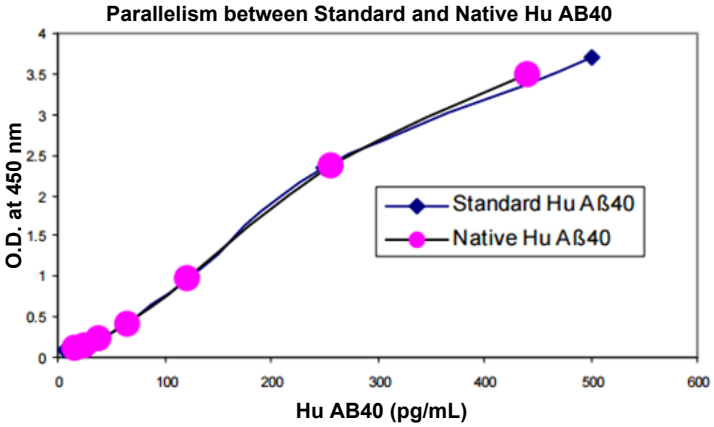
Linearity of dilution

Human CSF containing 195 pg/mL of measured human Aβ40 was serially diluted in Standard Diluent Buffer over the range of the assay. RPMI containing 10% fetal calf serum was spiked with the native human Aβ40 from CSF to a level of 212 pg/mL, and 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 0.99.

Dilution	Cerebrospinal Fluid			Cell Culture Supernatant		
	Measured (pg/mL)	Expected		Measured (pg/mL)	Expected	
		(pg/mL)	%		(pg/mL)	%
Neat	195	195	—	212	212	—
1/2	81	98	83	100	106	94
1/4	43	49	88	49	53	92
1/8	24	24	100	28	27	104

Parallelism

Native human Aβ40 was spiked into Standard Diluent Buffer and measured against the standard used in this kit. Parallelism between the two peptides is demonstrated by the figure below.



Recovery

The recovery of native human Aβ40 added to human CSF averaged 87%. The recovery of native human Aβ40 added to tissue culture medium containing 10% fetal calf serum averaged 98%. The recovery of native human Aβ40 added to 25% tissue homogenate averaged 92%.

Sensitivity

The analytical sensitivity of the assay is <6 pg/mL human Aβ40. 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 of 50 ng/mL were assayed in the Human Aβ40 ELISA Kit. The following substances were tested and found to have no cross-reactivity: Aβ [1-12], Aβ [1-20], Aβ [12-28], Aβ [22-35], Aβ [1-42], Aβ [1-43], Aβ [42-1], α-synuclein (90 ng/mL), APP (20 ng/mL), and Tau (10 ng/mL). **Rodent** β Amyloid (1-40) showed approximately 0.5% cross reaction at 50 ng/mL.

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.