Human Aβ42 Ultrasensitive ELISA Kit

Catalog nos. KHB3544

Pub. No. MAN0008231 **Rev** 2.0

Description

The Human Aβ42 Ultrasensitive (US) 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 amyloid 1-42) in tissue culture medium, tissue homogenates, cerebrospinal fluid (CSF), and other sample types. The assay will recognize both natural and synthetic forms of human Aβ42. The anti-human Aβ42 antibody used in this kit is capable of selectively detecting Aβ42 and not Aβ40/Aβ43.

Contents and storage

The components included in the ELISA kit are listed below. Upon receipt, store the kit at 2°C to 8°C.

Components	Cat. No. KHB3544 96 tests
Hu Aβ42 Antibody Coated Wells. 96 well plate.	1 plate
Hu Aβ42 US Detection Antibody. Contains 0.1% sodium azide; blue dye*	6 mL
Anti-Rabbit IgG HRP (100X). Contains 3.3 mM thymol.	0.125 mL
HRP Diluent. Contains 3.3 mM thymol, yellow dye*.	25 mL
Hu Aβ42 Standard. Lyophilized. Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	1 vial
Wash Buffer Concentrate (25X).	100 mL
Standard Diluent Buffer. Contains 0.1% sodium azide, red dye*.	60 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB).	25 mL
Stop Solution.	25 mL
Adhesive Plate Covers.	2

* 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.

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.

Materials required but not provided

- Distilled or deionized water
- $NaHCO_3$, ultrapure grade
- AEBSF (4-(2-aminoethyl)-benzenesulfonyl fluoride) or protease inhibitor cocktail containing AEBSF
- 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

General Guidelines

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com/techresources** for details prior to starting the procedure.
- Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

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Prepare 1X Wash Buffer

1. Allow the Wash Buffer Concentrate (25X) to reach room temperature and mix to dissolve any precipitated salts.
2. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
3. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Sample preparation guidelines

- 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.
- See the ELISA technical guide, available at thermofisher.com/techresources, for a procedure on homogenization of human or transgenic mouse brains.

Dilute samples

- Dilute samples up to 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.
- Because conditions may vary, it is recommended that each investigator determine the optimal dilution to be used for each application.

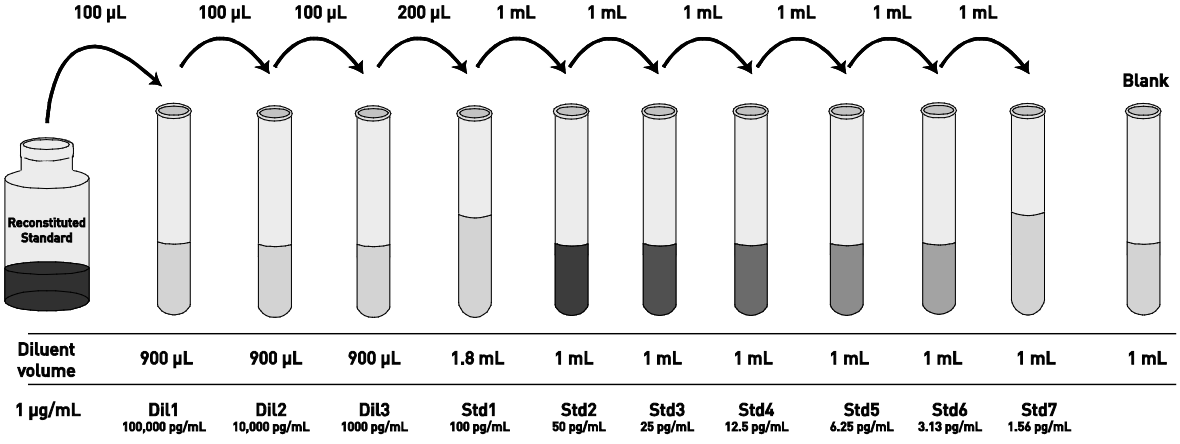
Prepare standard reconstitution buffer (55 mM sodium bicarbonate, pH 9.0)

1. Dissolve 2.31 grams of sodium bicarbonate in 500 mL of deionized water.
2. Add 2 N sodium hydroxide until pH is 9.0. Filter solution through a 0.2 µm filter unit.

Dilute standards

Note: Use plastic tubes for diluting standards. Standards must be diluted using the same composition of buffers used for the diluted experimental samples.

1. Reconstitute Hu Aβ42 Standard to 1 µg/mL with **standard reconstitution 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 1 µg/mL Hu Aβ42.
2. Add 900 µL Standard Diluent Buffer to each of 3 tubes labeled as follows: and label as 100,000, 10,000, and 1000 pg/mL Hu Aβ42.
3. Add 1.8 mL Standard Diluent Buffer to a tube and label as 100 pg/mL Hu Aβ42.
4. 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 Hu Aβ42.
5. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
6. Add AEBSF to diluted standards to a final concentration of 1 mM to prevent proteolysis of Aβ peptides.
7. The remaining reconstituted standard can be stored in aliquots at –80°C for up to 4 months, and can be frozen and thawed one time without loss of immunoreactivity. Return the Standard Diluent Buffer to the refrigerator.



Prepare secondary antibody solution

Note: Prepare the Anti-Rabbit IgG HRP secondary antibody solution within 15 minutes of usage.

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

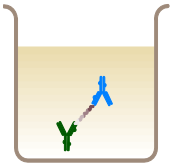
1. For each 8-well strip used in the assay, pipette 10 µL Anti-Rabbit IgG HRP (100X) solution, and wipe the outside of the pipette tip with a clean absorbent paper to remove any excess solution.
2. Dispense the solution to a tube containing 1 mL of HRP Diluent. Mix thoroughly.
3. Return any unused Anti-Rabbit IgG HRP (100X) solution to the refrigerator.

ELISA procedure

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 4 hours.**

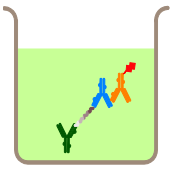
IMPORTANT! Perform a standard curve with each assay.

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 to 8°C for future use.



Bind antigen and add detector antibody

1. Add 50 µL of standards or diluted samples (see page 2) to the appropriate wells.
2. Add 50 µL of Detection Antibody solution into each well except chromogen blanks.
3. Cover the plate with plate cover and incubate for 3 hours at room temperature (or overnight at 4°C).
4. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.



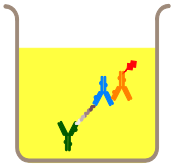
Add secondary antibody

5. Add 100 µL secondary antibody solution (see page 2) into each well except the chromogen blanks.
6. Cover the plate with plate cover and incubate for 30 minutes at room temperature.
7. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

Add chromogen

8. Add 100 µL Stabilized Chromogen to each well. The substrate solution will begin to turn blue.
9. Cover the plate with plate cover and incubate for 30 minutes at room temperature **in the dark**.

Note: TMB should not touch aluminum foil or other metals.



Add stop solution

10. Add 100 µL Stop Solution to each well. Tap side of the plate gently 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 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four 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 that of the highest standard in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

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

Standard Hu Aβ42 (pg/mL)	Optical Density (450nm)
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

Specificity

Buffered solutions of a panel of substances were assayed with the β amyloid 1-42 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).

Sensitivity

The minimum detectable dose of Hu 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.

Performance characteristics, continued

Intra-assay precision

Samples of known Hu 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
SD	5.76	3.75	1.78
%CV	8.04	9.37	8.33

SD = Standard Deviation; CV = Coefficient of Variation

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
SD	5.24	3.96	1.13
%CV	7.36	9.85	5.32

SD = Standard Deviation; CV = Coefficient of Variation

Recovery

The recovery of native Hu Aβ42 added to CSF and tissue culture medium containing 10% fetal bovine serum or 10% tissue homogenate was measured on the Hu Aβ42 (US) ELISA.

Sample	Average Recovery
CSF	106%
RPMI+10% fetal bovine serum	111%
10% Tissue homogenate	106%

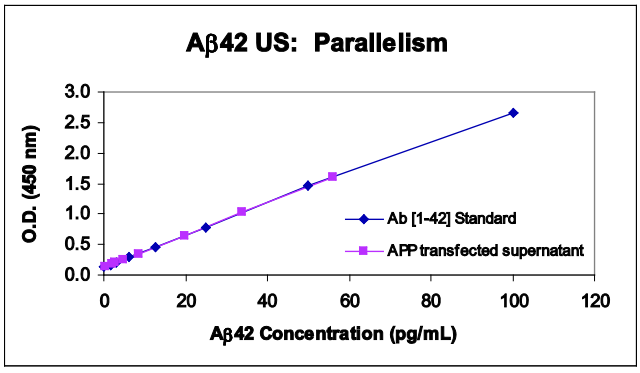
Linearity of dilution

Human CSF containing 280 pg/mL of measured 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 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 (pg/mL)	% Expected	Measured (pg/mL)	Expected (pg/mL)	% Expected
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

Parallelism

Native 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.



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.

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

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