Human Aβ42 ELISA Kit

Catalog nos. KHB3441
KHB3442

Pub. No. MAN0014629 **Rev**1.0

Description

The Human Aβ42 (Hu Aβ42) ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA) designed to detect and quantify the level of Hu Aβ42 in samples (e.g., tissue culture medium, tissue homogenate, cerebrospinal fluid (CSF), etc.) The assay will recognize both natural and synthetic forms of Hu Aβ42.

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. KHB3441 96 tests	Cat. no. KHB3442 192 tests
Aβ Antibody Coated Wells. 96 well plate.	1 plate	2 plates
Hu Aβ42 Detection Antibody. Contains 0.1% sodium azide, blue dye*.	6 mL	2 × 6 mL
Hu Aβ42 Standard. Lyophilized synthetic peptide. Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	1 vial	1 vial
Wash Buffer Concentrate (25X).	100 mL	100 mL
Standard Diluent Buffer. Contains 0.1% sodium azide, red dye*.	60 mL	2 × 60 mL
Anti-Rabbit IgG HRP (100X). Contains 3.3 mM thymol.	0.125 mL	2 × 0.125 mL
HRP Diluent. Contains 3.3 mM thymol, yellow dye*.	25 mL	25 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB).	25 mL	25 mL
Stop Solution.	25 mL	25 mL
Adhesive Plate Covers.	2	4

* 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
- 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.

Prepare 1X Wash Buffer

1. Allow the Wash Buffer Concentrate (25X) to reach room temperature and mix to redissolve 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.
- 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 helpful. Keep samples on ice until ready to apply to plate.

Dilute samples

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

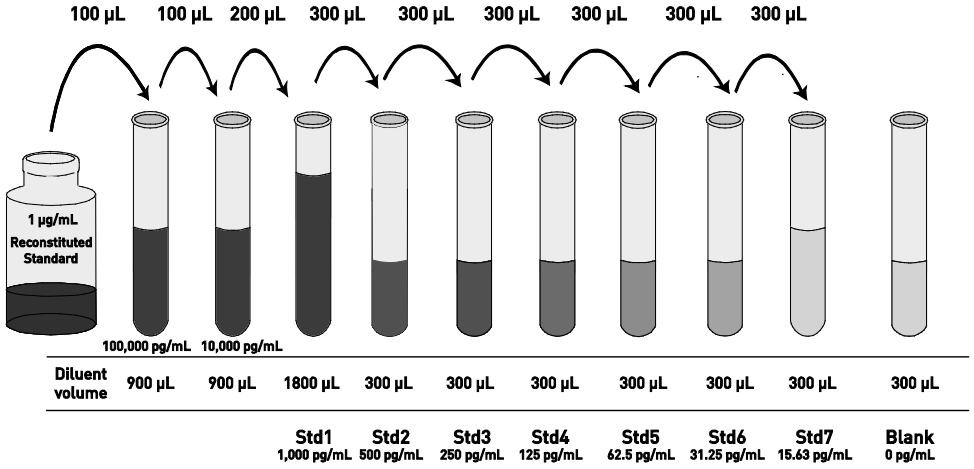
Dilute standards

Note: Polypropylene tubes may be used for standard dilutions. Hu Aβ42 Standard is calibrated against highly purified Hu Aβ where mass was corrected for peptide content by amino acid analysis.

1. Reconstitute Human Aβ42 Standard to 1 µg/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. **Use the standard within 1 hour of reconstitution**.

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).

2. Add 100 µL Reconstituted Standard to one tube containing 900 µL Standard Diluent Buffer and label as 100,000 pg/mL Hu Aβ42.
3. Add 100 µL of 100,000 pg/mL standard to one tube containing 900 µL Standard Diluent Buffer and label as 10,000 pg/mL Hu Aβ42.
4. Add 200 µL of 10,000 pg/mL standard to one tube containing containing 1,800 µL Standard Diluent Buffer and label as 1,000 pg/mL Hu Aβ42.
5. Add 300 µL Standard Diluent Buffer to each of 6 tubes labeled as follows: 500, 250, 125, 62.5, 31.35, and 15.63 pg/mL Hu Aβ42.
6. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
7. Discard remaining reconstituted standard or freeze in aliquots at -80°C for up to 4 months. Standard can be frozen and thawed one time only without loss of activity. Return Standard Diluent Buffer to the refrigerator.



Prepare 1X Anti-Rabbit IgG HRP solution

Note: Prepare 1X Anti-Rabbit IgG HRP solution within 15 minutes of usage.

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

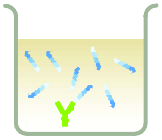
1. Allow Anti-Rabbit IgG HRP (100X) to reach room temperature and mix gently.
2. For each 8-well strip used in the assay, pipet 10 µL Anti-Rabbit IgG HRP (100X) solution, wipe the pipette tip with a clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of HRP Diluent. Mix thoroughly.
3. Return the 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.

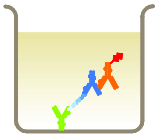


Bind antigen

1. Add 50 µL Standard Diluent Buffer to zero standard wells. Wells for chromogen blank should be left empty.
2. Prepare standards and samples with appropriate diluents. Add 50 µL of standards and diluted samples to the appropriate wells.

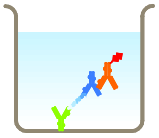
Add detector antibody

3. Add 50 µL Human Aβ42 Detection Antibody solution into each well except the chromogen blanks.
4. Tap the side of the plate to mix. Cover the plate with plate cover and incubate for 3 hours at room temperature with shaking.
5. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.



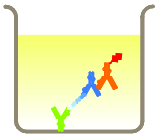
Add Anti-Rabbit IgG HRP

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



Add chromogen

9. Add 100 µL Stabilized Chromogen to each well. The substrate solution will begin to turn blue.
10. Incubate for 30 minutes at room temperature **in the dark**.
    Note: TMB should not touch aluminum foil or other metals.



Add stop solution

11. Add 100 µL of 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 30 minutes 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 0-1,000 pg/mL Hu Aβ42.

Standard Hu Aß42 (pg/mL)	Optical Density (450 nm)
1,000	3.06
500	1.85
250	1.02
125	0.51
62.5	0.27
31.25	0.19
15.63	0.15
0	0.20

Specificity

Buffered solutions of a panel of substances were assayed with the Aβ42 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] (100 ng/mL), Aβ [1-43] (10 ng/mL), Aβ [42-1] (100 ng/mL), α- Synuclein (200 ng/mL), APP (250 ng/mL), and Tau (40 ng/mL).

Recovery

The recovery of native Hu Aβ42 added to human CSF averaged 80%. The recovery of native Hu Aβ42 added to tissue culture medium containing 10% fetal calf serum averaged 86%.

Sensitivity

The minimum detectable dose of Hu Aβ42 is <10 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)	63.0	293.7	884.6
SD	3.1	7.8	26.7
%CV	5.0	2.7	3.0

SD = Standard Deviation; CV = Coefficient of Variation

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)	63.1	293.7	884.6
SD	3.5	9.3	36.6
%CV	5.5	3.2	4.1

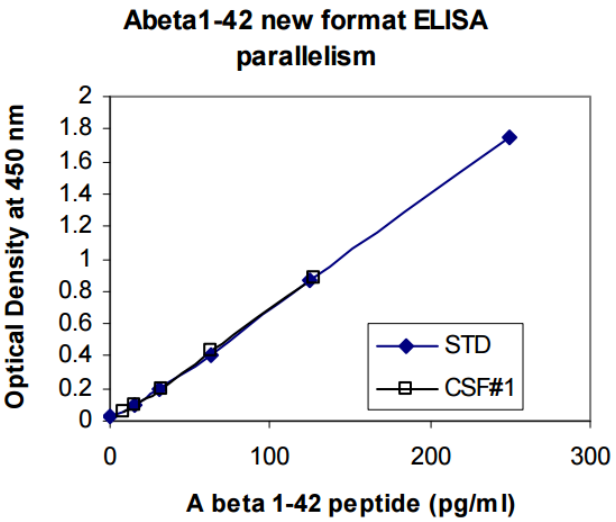
SD = Standard Deviation: CV = Coefficient of Variation

High Dose Hook Effect

Samples spiked with Hu Aβ42 peptide up to 100 ng/mL gave responses higher than that obtained for the last standard point.

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 by the figure below.



Linearity of dilution

Human CSF containing Aβ42 was serially diluted in Standard Diluent Buffer over the range of the assay. RPMI containing 10% fetal calf serum was spiked with the natural Aβ42 from APP transfected cells and 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		
	Measured (pg/mL)	Expected (pg/mL)	% Expected
1/4	127	127	—
1/8	71	63.5	112
1/16	37	31.8	116
1/32	18.7	15.9	118
1/64	7.6	8.0	96

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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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