

Product Information

β -Amyloid 1-42 ELISA, Human

hA β 42 ELISA

Catalog Number **BE0200**

Storage Temperature **2-8 °C**

Product Description

β -Amyloid 1-42 ELISA, Human is used for *in vitro* quantitative determination of human β -Amyloid 1-42 (hA β 42) protein in cell culture supernatants, tissue homogenates, cerebrospinal fluid (CSF), and other biological samples. The assay recognizes both natural and synthetic forms of hA β 42. The Anti-Human A β 42 antibody used in this kit is capable of selectively detecting A β 42 and not A β 40/A β 43.^{1,2}

The β -Amyloid 1-42 detection assay is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for the NH₂ terminus of hA β has been coated onto the wells of the multiwell plate provided. Standards of known hA β 42 content, control specimens and unknown samples are pipetted into these wells and co-incubated with a rabbit antibody specific for the 1-42 sequence of human A β . During the first incubation, hA β antigen binds to the immobilized (capture) antibody and the detection antibody binds to the bound hA β 42 protein. Bound rabbit antibody is detected by the use of Anti-Rabbit IgG-Horseradish Peroxidase. After a second incubation and washing to remove all the excess Anti-Rabbit IgG-HRP, a substrate solution is added, which is acted upon by the bound enzyme to produce yellow color. The intensity of this colored product is directly proportional to the concentration of hA β 42 present in the original specimen. The optical density measured at 450 nm in a multiwell plate reader is used to calculate the concentration of hA β 42.

This assay has been calibrated against the mass determination of highly purified native A β where mass was corrected for peptide content by amino acid analysis. Researchers may also use β Amyloid 1-40 ELISA, Catalog Number BE0100, to detect hA β 40.

Alzheimer's Disease (AD) is characterized by the presence of extracellular plaques and intracellular

neurofibrillary tangles (NFTs) in the brain.¹⁻⁵ The major protein component of these plaques is β amyloid peptide (A β), a 40 to 43 amino acid peptide cleaved from amyloid precursor protein by β -secretase (e.g. BACE) and a putative γ -secretase. Increased release of the 'longer forms' of A β peptide, A β 42 or A β 43, which have a greater tendency to aggregate than A β 40, occurs in individuals expressing certain genetic mutations, ApoE alleles, or may involve other, still undiscovered, factors. Many researchers theorize that it is this increased release of A β 42/A β 43 that leads to the abnormal deposition of A β and the associated neurotoxicity in the brains of affected individuals. In patients with AD, reduced levels of A β 42 in CSF have been described as predictive of AD.⁶

Reagents

- **β -Amyloid 1-42 Standard, Human, 1 vial, Catalog Number A1853** – Lyophilized synthetic peptide. Refer to vial label for quantity and reconstitution volume.
- **Standard/Sample Diluent, 60 mL, Catalog Number S5568** – contains BSA and 15 nM sodium azide as preservative. Contains a red dye. Ready to use.
- **Monoclonal Anti- Human A β -coated 96 well plate, 1 plate, Catalog Number A1103** - A plate, using break-apart strips, coated with monoclonal antibody specific for NH₂ terminus of hA β .
- **Anti-Human β -Amyloid 1-42, 6 mL, Catalog Number A1728** – A detection antibody, produced in rabbit, which recognizes a human A β 42. Contains 15 mM sodium azide and a blue dye.
- **Anti-Rabbit IgG-HRP Concentrate (100x), 1 vial, 0.125 mL, Catalog Number I5033** – contains 3.3 mM thymol and 50% glycerol. See **Preparation Instructions** for handling, dilution and storage instructions.
- **HRP Diluent, 25 mL, Catalog Number I5158** – contains 3.3 mM thymol, BSA and a yellow dye.. Ready to use.

- **Wash Buffer Concentrate 25x, 100 ml, Catalog Number W2639** - See **Preparation Instructions** for handling, dilution and storage instructions.
- **Stabilized Chromogen (TMB), 25 mL, Catalog Number S3318** –Avoid prolonged exposure to light. Avoid exposure to metal. Ready to use.
- **Stop Solution, 25 mL, Catalog Number S2818** – Ready to use.
- **Plate Covers, Adhesive strips, 3 each, Catalog Number P4870**

Reagents and Equipment required but not provided

- Standard Reconstitution Buffer - 55 mM NaHCO₃, ultrapure grade, Catalog Number S6297, pH 9.0.
- Multiwell plate reader capable of readings at 450 nm.
- Calibrated adjustable precision pipettes for volumes between 5 µL and 1,000 µL.
- Reagent reservoirs for dispensing standards, antibodies and substrate.
- 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), Catalog Number A8456, or protease inhibitor cocktail containing AEBSF, Catalog Number P8340).
- Deionized or distilled water.
- Plate washer (optional), use squirt bottle, manifold dispenser, etc.
- Graph paper: linear, log-log, or semi-log, as desired
- Disposable polypropylene 12x75 mm tubes for diluting reagents, standards and samples.
- Absorbent paper towels to blot the plate
- Calibrated beakers and graduated cylinders in various sizes
- Vortex mixer
- Shaking platform or mini-orbital shaker

Precautions and Disclaimer

The kit is for R&D use only, not for drug, household or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

- Samples of choice: cell culture media and other biological fluids.
- Samples should be separated from the cells and frozen if not analyzed shortly after collection.
- Dilute samples 1:2 to 1:10 in media or Standard/Sample Diluent.

- Sample matrix has a dramatic impact on A β recovery. To ensure accurate quantitation, the standard curves and blanks must be generated in the same diluent as the samples.
- Serine proteases can rapidly degrade A β peptides, thus, using AEBSF protease inhibitor (water soluble and less toxic than PMSF) at a 1 mM final concentration stabilizes the A β . Add AEBSF to all samples before applying to the plate.
- Analysis of plasma samples may require pretreatment to disrupt binding of A β to masking proteins.
- Keep samples on ice until ready to apply to plate.
- Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
- When possible avoid use of hemolyzed or lipemic samples.
- Make sample dilutions in polypropylene tubes.
- Avoid use of sodium azide, thymol or thimerosal as sample preservatives.

Reagent Preparation

Human β -Amyloid 1-42 Standard

1. Prepare Standard Reconstitution buffer (not supplied). Dissolve 2.31 g of sodium bicarbonate in 500 ml of distilled or deionized water. Add 2N sodium hydroxide until pH is 9.0. Filter buffer through a 0.2 µM filter. The solution is sterile.
2. Polypropylene tubes may be used for standard reconstitution and dilutions.
3. Reconstitute the hA β 42 Standard to 1.0 µg/mL with Standard Reconstitution Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow vial to sit for 5 minutes at room temperature. Briefly vortex prior to preparing standards.
4. Standard curve of the hA β 42 standards provided in the kit must be generated using the same composition of buffers used for the diluted experimental samples. For example, if brain extracts are diluted 1:10 with Standard/Sample Diluent, then the buffer used to dilute standards should be 90% Standard/Sample Diluent and 10% brain extraction buffer (including AEBSF at a final concentration of 1 mM).

Prepare serial standard dilutions as follows

Tube #	Standard/ Sample Diluent or Sample matrix	Standard from tube #:	Final hA β 42-pg/ml
A	Reconstitute Standard according to label instructions		1 μ g/ml
B	0.900 ml	0.1 ml (A)	100,000 pg/ml
C	0.900 ml	0.1 ml (B)	10,000 pg/ml
1	1.8 ml	0.2 ml (C)	1000 pg/ml
2	1 ml	1 ml (1)	500 pg/ml
3	1 ml	1 ml (2)	250 pg/ml
4	1 ml	1 ml (3)	125 pg/ml
5	1 ml	1 ml (4)	62.5 pg/ml
6	1 ml	1 ml (5)	31.2 pg/ml
7	1 ml	1 ml (6)	15.63 pg/ml
8	1 ml	-	0 pg/ml

Mix thoroughly between steps.

- Use within 1 hour of reconstitution.

Anti-Rabbit IgG Horseradish Peroxidase (HRP), 100x Concentrate

Contains 50% glycerol and is very viscous. To ensure accurate dilution follow instructions below:

- Equilibrate to room temperature, mix gently, pipette slowly.
- Remove excess concentrate solution from pipette tip with clean absorbent paper.
- Mix 10 μ l IgG-HRP concentrate +1 ml HRP Diluent (sufficient for one 8-well strip, prepare more if needed as in table below)
Label as **Anti-Rabbit IgG-HRP Working Solution**.
- Store the unused Anti-Rabbit IgG-HRP Concentrate at 2-8 °C

# of 8-well strips	Antibody (100X concentrate) mL	HRP Diluent mL
2	20 μ L	2 mL
4	40 μ L	4 mL
6	60 μ L	6 mL
8	80 μ L	8 mL
10	100 μ L	10 mL
12	120 μ L	12 mL

Wash Buffer

- Equilibrate the 25x concentrate to room temperature and mix to redissolve any precipitated salts.
- Mix 1 volume Wash Buffer Concentrate 25x + 24 volumes of deionized water
- Label as **Working Wash Buffer**.
- Store both the concentrate and the Working Wash Buffer in the refrigerator. Use within 14 days.

Storage/Stability

All components of this kit are stable at 2-8 °C. Any unused reconstituted standard should be discarded or frozen at -70 °C. Standard can be frozen and thawed one time only without loss of immunoreactivity.

Refer to the Certificate of Analysis for kit expiration date. To obtain C of A go to www.sigma-aldrich.com

Procedure

Precautions

- 20-30 minutes before use equilibrate kit and all reagents to room temperature (15-30 °C).
- Use only the coated 96 well capture plate provided with the kit.
- Multiwell plate: equilibrate to room temperature in unopened foil bag. Remove desired number of strips, reseal the bag and refrigerate at 2-8 °C to maintain plate integrity.
- When not in use all kit components should be refrigerated.
- Assay all standards, controls and samples in duplicate.
- If particulate matter is present, centrifuge or filter prior to analysis.
- A standard curve must be run with each assay.
- Maintain a consistent order of component and reagent addition from well to well. This ensures equal incubation times for all wells.
- Run in-house controls with every assay. If control values fall outside pre-established ranges, the accuracy of the assay may be suspect.
- All reagents are lot-specific. Do not mix reagents from different kit lots.
- Do not use reagents after the kit expiration date.
- Use polypropylene tubes to dilute standards and samples.
- Pre-rinse the pipette tip with the reagent and use fresh pipette tips for each sample, standard or reagent.
- Read absorbance within 2 hours of assay completion.

Washing directions

- The purpose of washing is to remove unbound proteins and other non-specific parts in solution.
- Incomplete washing will adversely affect the assay and render false results.
- Use only Wash Buffer provided in kit.
- Washing may be performed using automated washer, manifold pipette or squirt bottle.
- Wash cycle four times, blotting as dry as possible after the 4th wash.
- When washing manually, fill wells with Wash Buffer, aspirate thoroughly and tap dry on absorbent tissue.
- It is recommended to use laboratory tape to hold plate strips to the plate frame while performing the plate washing and drying procedure to avoid strips coming free of the frame.

hA β 42 Assay Summary**1. 1st incubation****50mL of standards and samples****50 mL detection antibody****Incubate 3 hours at RT while shaking, or overnight at 2-8 °C without shaking.****Aspirate and wash 4x****2. 2nd incubation****100 mL of Anti-Rabbit IgG-HRP****Incubate 30 minutes at RT.****Aspirate and wash 4x****3. 3rd incubation****100 mL of stabilized Chromogen****Incubate 30 minutes at RT (*in the dark*).****4. Stop step****100 mL of Stop Solution****Read at 450 nm****Total Time 4 hours**

- Determine the number of wells for the assay run, including 2 zero wells, 2 chromogen blank wells, 14 standard dilutions wells and 2 wells for each sample to be assayed.
- Remove appropriate number of multiwell strips and return the unused strips to the pouch. Reseal pouch.

1st incubation

- Add 50 μ L/well Standard Diluent to zero wells.
- Add 50 μ L/well standards, samples or controls to the appropriate wells.
- Add 50 μ L of detection antibody to all the wells
- Tap gently on the plate to mix, cover with Plate Cover and incubate 3 hours, while shaking on the platform at room temperature. Alternatively, plate may be incubated overnight at 2-8 °C without shaking.
- Wash wells 4 times following washing directions.
- After the final wash blot dry on a lint free paper towel to remove any remaining wash buffer

2nd incubation

- Add 100 μ L Anti-Rabbit IgG-HRP Working Solution to each well except the chromogen blanks.
- Cover with Plate Cover and incubate 30 minutes at room temperature
- Wash wells for a total of 4 times following washing instructions.
- After the final wash blot dry on a lint free paper towel to remove any remaining wash buffer.

Substrate incubation

- Add 100 μ L of Stabilized Chromogen into all wells. The liquid in the wells will begin to turn blue.
- Do not cover the plate
- Incubate approximately 30 minutes at room temperature in the dark (place plate in a drawer or cabinet).

Note: If your multiwell reader does not register optical density (OD) above 2.0, incubate only 20-25 minutes.

Stop step

- Add 100 μ L of Stop Solution to each well. This stops the reaction
- Tap gently to mix. The solution will turn yellow.

Absorbance reading

- Any commercially available multiwell plate reader capable of reading at OD 450 nm may be used.
- Blank the plate reader against the Chromogen Blank wells (containing Chromogen and Stop Solution).
- Read the absorbance of the entire plate at 450 nm within 2 hours after addition of Stop Solution.

Results

The results may be calculated using any immunoassay software package. The four-parameter algorithm provides the best curve fit. If the software program is not readily available, the concentrations of β -Amyloid 1-42 may be calculated as follows:

1. Calculate the Average Net OD for each standard dilution and samples as follows:

$$\text{Average Net OD} = \text{Average Bound OD} - \text{Average Chromogen Blank OD}$$

2. On graph paper plot the Average Net OD of standard dilutions against the concentration (pg/ml) of hA β 42 for the standards. Draw the best curve through these points to construct the standard curve.
3. The hA β 42 concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
4. Multiply the values obtained for the samples by dilution factor of each sample.
5. Samples producing signals higher than the 1,000 pg/ml standard should be further diluted and assayed.

Product Profile

Typical Results

The standard curve below is for illustration only and **should not be used** to calculate results in your assay. Run standard curve in each assay.

Standard hA β 42 (pg/ml)	OD 450 nm
0	0.119
15.63	0.146
31.25	0.187
62.5	0.266
125	0.510
250	1.018
500	1.848
1000	3.060

Limitations:

- Do not extrapolate the standard curve beyond the 1,000 pg/ml standard point.
- The dose response is non-linear in this region and accuracy will be compromised.
- Other buffers and matrices have not been investigated.

- The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced, etc.) and the use of biological fluids in place of serum samples have not been thoroughly investigated.
- The rate of degradation of native hA β in various matrices has not been investigated.
- The immunoassay literature contains frequent references to aberrant signals seen with some sera, attributed to heterophilic antibodies. Though such samples have not been seen to date, the possibility of this occurrence cannot be excluded.

Performance Characteristics

Specificity

Buffered solutions of a panel of substances were assayed with the β Amyloid 1-42 Elisa Kit. The following substances were tested and found to have no cross-reactivity: A β [1-12] (100 ng/mL); A β [1-20] (100 ng/mL); A β [12-28] (100 ng/mL); A β [22-35] (100 ng/mL); A β [1-40] (100 ng/mL); A β [1-43] (10 ng/mL); A β [42-1] (100 ng/mL); a-Synuclein (200 ng/mL); APP (250 ng/mL); and Tau (40 ng/mL).

Sensitivity

Sensitivity of this assay is <10 pg/ml.

Sensitivity was calculated by adding two standard deviations to the mean OD obtained when the zero standard was assayed 64 times.

Precision

1. Intra-Assay Precision

Samples of known hA β 42 concentrations were assayed in replicates of 16 to determine precision within an assay.

	Sample 1	Sample 2	Sample 3
Mean (pg/ml)	884.6	293.7	63.0
Standard Deviation (SD)	26.7	7.8	3.1
% Coefficient of Variation	3.0	2.7	5.0

2. Inter-Assay Precision

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

	Sample 1	Sample 2	Sample 3
Mean (pg/ml)	884.6	293.7	63.1
Standard Deviation (SD)	36.6	9.3	3.5
% Coefficient of Variation	4.1	3.2	5.5

Linearity of Dilution

Human CSF containing 304 pg/mL of measured β Amyloid 1-42 was serially diluted in Standard/Sample Diluent over the range of the assay. RPMI containing 10% fetal bovine serum was spiked with natural β amyloid 1-42 from APP transfected cells to a level of 1364 pg/mL, 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 in both cases.

CSF			
Dilution	Measured (pg/ml)	Expected (pg/mL)	% Expected
1:4	341.0	341.0	
1:8	185.4	170.5	108%
1:16	94.6	85.3	107%
1:32	41.2	42.6	97%

Cell Culture Supernatant			
Dilution	Measured (pg/ml)	Expected (pg/mL)	% Expected
1:2	152.1	152.1	-
1:4	76.4	76.0	101
1:8	38.4	38.0	101
1:16	15.2	19.0	80

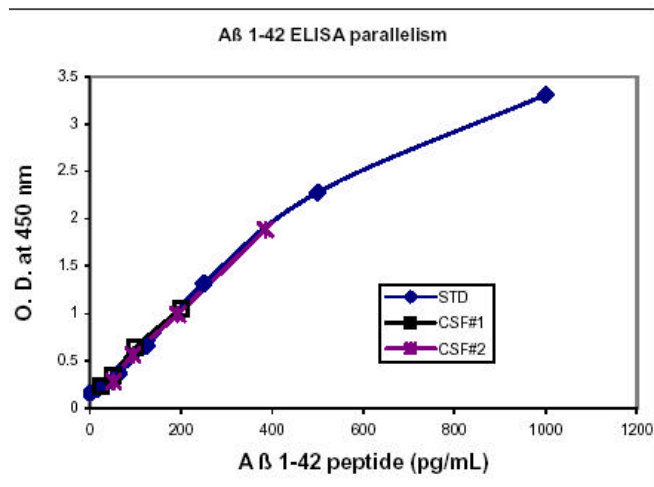
Recovery

The recovery of native hA β 42 added to human CSF averaged 116%. The recovery of native hA β 42 added to tissue culture medium containing 10% fetal calf serum averaged 90%.

Parallelism

Native A β 42 was serially diluted in Standard/Sample Diluent and measured against the standard used in this kit.

Parallelism between the two peptides is demonstrated by the figure below:



References

1. Citron, M., et al., β -secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic Protease BACE. *Science*, **286**, 735-741 (1999).
2. Savage, M.J., et al., Turnover of β -amyloid protein in mouse brain and acute reduction of its level by phorbol ester. *J. Neurosci.*, **18**, 1743-1752 (1998).
3. Bancher, C., et al., Mechanisms of cell death in Alzheimer's disease. *J. Neural Transm. Suppl.*, **50**, 141-152 (1997).
4. Lorenzo, A., et al., Amyloid β interacts with the amyloid precursor protein: a potential toxic mechanism in Alzheimer's disease. *Nat. Neurosci.*, **3**, 460-464 (2000).
5. Sambamurti, K., et al., Advances in the cellular and molecular biology of the β -amyloid protein in Alzheimer's disease. *Neuromolecular Med.*, **1**, 1-31 (2002).
6. Andreasen, N., et al., Cerebrospinal fluid β -amyloid (1-42) in Alzheimer disease: differences between early- and late-onset Alzheimer disease and stability during the course of disease. *Arch. Neurol.*, **56**, 673-680 (1999).
7. Kuo, Y. M., et al., Amyloid β peptides interact with plasma proteins and erythrocytes: implications for their quantitation in plasma. *Biochem. Biophys. Res. Commun.*, **268**, 750-756 (2000).

Appendix

β -Amyloid Application: Procedure for homogenization of human or transgenic mouse brains

For Tissue Homogenization, Prepare the Following Solutions:

- A. 5 M guanidine HCl
50 mM Tris HCl, pH 8.0
- B. Reaction Buffer BSAT-DPBS (Dulbecco's phosphate buffered saline with 5% BSA and 0.03% Tween[®] 20, see formulation below) supplemented with 1x Protease Inhibitor Cocktail, (containing aprotinin, E64, EDTA, and leupeptin).

BSAT-DPBS Formulation

0.2 g/L KCl
0.2 g/L KH₂PO₄
8.0 g/L NaCl
1.150 g/L Na₂HPO₄
5% BSA
0.03% Tween 20
q.s. to 1 L with ultrapure water and adjust the pH to 7.4.

Protocol:

- Determine the wet mass of the mouse hemibrain (100 mg) or a human brain sample in an Eppendorf tube.
- Add 8x mass of cold Solution A (5 M guanidine HCl / 50 mM Tris HCl) to the tube in 50 - 100 μ L aliquots and grind thoroughly with a hand-held motor after each addition.
Optional: transfer the homogenate from above to a 1 mL Dounce homogenizer and homogenize thoroughly.)

- Mix the homogenate at room temperature for 3 - 4 hours. The sample is stable and can be freeze-thawed many times at this stage.
- Dilute the sample with cold Reaction Buffer (Solution "B", above). Centrifuge (microfuge) at 16,000 x g for 20 minutes at 4°C. This dilution factor requires adjustment depending on the quantity of A β present and on inhibition of the standard curve development due to the presence of guanidine. Initial experiments indicate a dilution factor of 1:200 for human brain and 1:20 to 1:50 for transgenic mouse brains. The optimal dilution factor should be determined for each specific experimental determination.
Note: we have determined that the standard curve can withstand the presence of 0.1 M or less guanidine solution. Inclusion of guanidine at a concentration higher than 0.1 M will result in significant depression of the standard curve.
- Carefully decant the supernatant and store on ice until use with the β -Amyloid 1-42 ELISA

Alternative Procedure :

Homogenization can be performed with 4x volume of cold PBS supplemented with the 1x protease inhibitor cocktail, followed by the addition of a solution 8.2 M guanidine / 82 mM Tris HCl (pH 8.0) to yield a solution with 5 M final guanidine concentration.

References for Homogenization Procedure:

- Maslah, E., et al. β -amyloid peptides enhance α -synuclein accumulation and neuronal deficits in a transgenic mouse model inking Alzheimer's disease and Parkinson's disease. Proc. Nat'l. Acad. Sci. **98**, 12245-12250 (2001).
- Johnson-Wood, K., et al., Amyloid precursor protein processing and A β 42 deposition in a transgenic mouse model of Alzheimer disease. Proc. Nat'l. Acad. Sci. **94**, 1550-1555 (1997).
- Chishti, M.A., et al. Early-onset amyloid deposition and cognitive defects in transgenic mice expressing a double mutant form of amyloid precursor protein 695. J. Biol. Chem. **276**, 21562-21570 (2001).

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