ELISA Kit
Catalog #KHB3491

Human
Aggregated Beta Amyloid

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INTRODUCTION

Alzheimer's disease is characterized by the presence of numerous extracellular plaques (also known as senile plaques), composed primarily of beta amyloid (Aβ), as well as numerous intracellular neurofibrillary tangles, composed primarily of hyperphosphorylated tau protein. Associated with the extracellular plaques are activated microglia and astrocytes. Inflammatory processes, including enhanced cytokine production, Cox-2 activity, and PGE2 production, dysfunctional calcium transport, and production of free radicals can also be detected within brain tissue bearing plaques.

Mutations in amyloid precursor protein, PS1, PS2, and ApoE are observed in familial Alzheimer's disease. These mutations contribute to the enhanced rate of production and/or accumulation of Aβ. Aβ has therefore been suggested to play a key role in the etiology of Alzheimer's disease.

By atomic force microscopy and electron microscopy, Aβ can be observed to self-assemble into fibrils, protofibrils, and globular oligomeric structures. When applied to cultured neurons in its monomeric form, the peptide has little effect on the cells. However, when monomeric Aβ is treated in a manner that stimulates the formation of the self-assembled structures (procedures that usually involve dissolving the peptide in an organic solvent, then incubating the peptide in physiological solution at 37°C), the peptide becomes neurotoxic.

Several recent reports indicate that an important neurotoxic form of Aβ is an oligomer, composed of approximately 12 subunits, with a composite molecular weight of 54 kDa. This oligomeric form of Aβ (also known as Amyloid Derived Diffusible Ligand or ADDL) can be

separated from fibrillar and protofibrillar forms of aggregated beta amyloid by high speed centrifugation (i.e., 100,000 x g for 1 hour) or by size exclusion methods. The neurotoxicity of Aβ aggregates can be detected by measuring decreases in the rate of MTT reduction when applied to cells in culture. Aβ aggregates also stimulate the loss of synapses ("synaptosis") when applied to nerve terminals, a process which may lead to the decrease in hippocampal long term potentiation that is believed to underlie the memory loss associated with Alzheimer's disease.

The Invitrogen Human Aggregated Aβ ELISA is designed to detect and quantify the level of aggregated Aβ. This assay is intended for the detection of human aggregated Aβ but does not recognize mouse or rat.

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READ ENTIRE PROTOCOL BEFORE USE

PRINCIPLE OF THE METHOD

The Invitrogen Aggregated Aβ kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for the N-terminus of human Aβ has been coated onto the wells of the microtiter strips provided. Samples, including a standard containing aggregated Aβ, control specimens, and unknowns, are pipetted into these wells. During the first incubation, the Aβ binds to the immobilized (capture) antibody. After washing, biotinylated detector antibody (same monoclonal antibody used as the capture) is pipetted into the wells. During the second incubation, this antibody serves as a detection antibody by binding to the immobilized aggregated Aβ captured during the first incubation. After removal of excess detection antibody, a horseradish peroxidase-labeled streptavidin (SAV-HRP) is added. This binds to the detection antibody to complete the four-member sandwich. Four member solid phase sandwiches are formed only when analyte containing multiple copies (Aβ aggregates $\geq$2) of the Aβ N-terminus is bound. After a third incubation and washing to remove all the excess SAV-HRP, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of aggregated Aβ present in the original specimen.

REAGENTS PROVIDED

Note: *Store all reagents at 2 to 8°C.*

Reagent	***96 Test Kit***
Hu Aggregated Aβ Standard: Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials
Standard Diluent Buffer. Contains 0.1% sodium azide; red dye*; 60 mL per bottle.	1 bottle
*Antibody Coated Well*s. 12 x 8 Well Strips.	1 plate
Hu Aggregated Aβ Biotin Conjugate. Contains 0.1% sodium azide; blue dye*; 11 mL per bottle.	1 bottle
Streptavidin-HRP (100X). Contains 3.3 mM thymol; 0.125 mL per vial.	1 vial
HRP Diluent. Contains 3.3 mM thymol; yellow dye*; 25 mL per bottle.	1 bottle
Wash Buffer Concentrate (25X). 100 mL per bottle.	1 bottle
*Stabilized Chromoge*n, *Tetramethylbenzidine (TMB);* 25 mL per bottle.	1 bottle
*Stop Solutio*n; 25 mL per bottle.	1 bottle
Plate Covers, adhesive strips.	3
* In order to help our customers avoid any mistakes in pipetting the ELISAs, we provide colored *Standard Diluent Buffer*, *Detection Antibody*, and *HRP Diluent* to help monitor the addition of solutions to the reaction wells. This does not in any way interfere with the test results.	

Disposal Note: 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.

SUPPLIES REQUIRED BUT NOT PROVIDED

1. Microtiter plate reader capable of measurement at or near 450 nm.
2. Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)
3. Tissue extraction buffer (see Recommended Formulation, p. 12).
4. Distilled or deionized water.
5. Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
6. Data analysis and graphing software. Graph paper: linear (Cartesian), log-log, or semi-log, as desired.
7. Glass or plastic tubes for diluting and aliquoting standard.
8. Absorbent paper towels.
9. Calibrated beakers and graduated cylinders in various sizes.

PROCEDURAL NOTES/LAB QUALITY CONTROL

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. **Microtiter plates should be allowed to come to room temperature before opening the foil bags.** Once the desired number of strips has been removed, immediately reseal the bag and store at 2 to 8°C to maintain plate integrity.

3. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
4. If particulate matter is present, centrifuge or filter prior to analysis.
5. All standards, controls and samples should be run in duplicate.
6. Samples that are greater than the highest standard point should be diluted with *Standard Diluent Buffer* and retested.
7. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
8. Cover or cap all reagents when not in use.
9. **Do not mix or interchange different reagent lots from various kit lots**.
10. Do not use reagents after the kit expiration date.
11. Read absorbances within 2 hours of assay completion.
12. In-house controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
13. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. ***Never*** insert absorbent paper directly into the wells.
14. Because *Stabilized Chromogen* is light sensitive, avoid prolonged exposure to light. Also avoid contact between *Stabilized Chromogen* and metal, or color may develop.

SAFETY

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

DIRECTIONS FOR WASHING

Incomplete washing will adversely affect the test outcome. All washing must be performed with *Wash Buffer Concentrate (25X)* provided.

Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip (aspiration device) into the bottom of each well. Take care not to scratch the inside of the well.

After aspiration, fill the wells with at least 0.4 mL of diluted wash solution. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under **ASSAY METHOD**. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

If using an automated washer, the operating instructions for washing equipment should be carefully followed. If your automated washer allows, 30 second soak cycles should be programmed into the wash cycle.

PREPARATION AND EXTRACTION OF AGGREGATED Aβ

A. Preparation of Sample

1. Aggregated Aβ has been detected in tissue homogenates, ventricular fluid, CSF, tissue culture supernatant, and buffered solution samples using the reagents provided in this kit.
2. Samples should be collected in pyrogen/endotoxin-free polypropylene tubes.
3. All samples should be clarified by centrifugation prior to analysis.
4. Due to shared epitopes, the following analytes may cause interference with this assay: APP, monomeric Aβ40, monomeric Aβ42, protofibrillar Aβ, and fibrillar Aβ. Sample preparation should therefore be carefully considered when using this assay. Centrifugation at 14,000 x g for 10 minutes has been shown to minimize fibrils in aggregated Aβ-containing samples, while centrifugation at 100,000 x g for 1 hour at 4°C has been shown to minimize fibrils and protofibrils. Size exclusion methods, such as gel permeation chromatography or ultrafiltration, may also improve assay performance.
5. Aggregated Aβ is sensitive to freeze-thaw cycles. **Avoid multiple freeze-thaw cycles of frozen samples.** Thaw completely and mix well prior to analysis.
6. Samples should be diluted at least 1:2 in *Standard Diluent Buffer* prior to analysis using the Aggregated Aβ ELISA.

B. Extraction of Aggregated Aβ from Tissue

Note: The procedure below provides guidelines for extraction of tissue samples. This procedure may require optimization for each specific application.

Recommended Tissue Extraction Buffer (Tris Buffered Saline)
25 mM Tris-HCl, pH 7.4
150 mM NaCl

This tissue extraction buffer may be stored at 2 to 8°C for up to 1 month. For maximal stability, apportion the tissue extraction buffer into aliquots and store at –20°C.

This buffer should be freshly supplemented with protease inhibitors just prior to use. Suggested protease inhibitors: 1.46 µM pepstatin A, 0.154 µM aprotinin, 2.03 µM leupeptin, 0.5 mM 4-[2-aminoethyl] benzenesulfonyl fluoride hydrochloride [AEBSF], and 0.29 mM PMSF. If desired, this buffer may also be supplemented with phosphatase inhibitors. Suggested phosphatase inhibitors: 0.05 mM fenvalerate, 0.05 mM cantharidin, 1 mM sodium vanadate, 1 mM sodium pyrophosphate, and 50 mM sodium fluoride.

1. Determine the mass of the tissue.
2. Add 10x volume tissue extraction buffer. Homogenize the tissue thoroughly with a hand-held motor (e.g., Fisher K749540-0000).
3. Sonicate the homogenized tissue (2 blasts, 10 seconds each).
4. Centrifuge the samples at 100,000 x g for 1 hour at 4°C. Carefully withdraw the clear supernatant fraction, and transfer to a clean polypropylene tube on ice. This supernatant fraction contains TBS-soluble aggregates of Aβ.
5. Samples should be diluted at least 1:2 in *Standard Diluent Buffer* prior to analysis using the Aggregated Aβ ELISA.

REAGENT PREPARATION AND STORAGE

A. Reconstitution and Dilution of Hu Aggregated Aβ Standard

1. Reconstitute *Hu Aggregated Aβ Standard* with *Standard Diluent Buffer*. Refer to standard vial label for instructions. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Label as 5.7 ng/mL Aggregated Aβ. Use the standard within 1 hour of reconstitution.
2. Add 0.25 mL of *Standard Diluent Buffer* to each of 6 tubes labeled 2.9, 1.4, 0.71, 0.36, 0.18 and 0.09 ng/mL Aggregated Aβ.
3. Make serial dilutions of the standard as described in the following dilution table. Mix thoroughly between steps.

B. Dilution of Hu Aggregated Aβ Standard

Standard:	Add:	Into:
5.7 ng/mL	Prepare as described in step 1	
2.9 ng/mL	0.25 mL of the 5.7 ng/mL std.	0.25 mL of the Diluent Buffer
1.4 ng/mL	0.25 mL of the 2.9 ng/mL std.	0.25 mL of the Diluent Buffer
0.71 ng/mL	0.25 mL of the 1.4 ng/mL std.	0.25 mL of the Diluent Buffer
0.36 ng/mL	0.25 mL of the 0.71 ng/mL std.	0.25 mL of the Diluent Buffer
0.18 ng/mL	0.25 mL of the 0.36 ng/mL std.	0.25 mL of the Diluent Buffer
0.09 ng/mL	0.25 mL of the 0.18 ng/mL std.	0.25 mL of the Diluent Buffer
0 ng/mL	0.25 mL of the Diluent Buffer	An empty tube

Remaining reconstituted standard should be discarded after use.

C. Storage and Final Dilution of Streptavidin-HRP (100X)

Please Note: The *Streptavidin-HRP (100X)* is in 50% glycerol. This solution is viscous. To ensure accurate dilution, allow *Streptavidin-HRP (100X)* to reach room temperature. Gently mix. Pipette *Streptavidin-HRP (100X)* slowly. Remove excess concentrate solution from pipette tip by gently wiping with clean absorbent paper.

1. Dilute 10 µL of this 100X concentrated solution with 1 mL of *HRP Diluent* for each 8-well strip used in the assay. Label as SAV-HRP Working Solution.

For Example:

# of 8-Well Strips	Volume of Streptavidin-HRP (100X)	Volume of Diluent
2	20 µL solution	2 mL
4	40 µL solution	4 mL
6	60 µL solution	6 mL
8	80 µL solution	8 mL
10	100 µL solution	10 mL
12	120 µL solution	12 mL

2. Return the unused *Streptavidin-HRP (100X)* to the refrigerator.

D. Dilution of Wash Buffer

Allow the *Wash Buffer Concentrate (25X)* to reach room temperature and mix to ensure that any precipitated salts have redissolved. Dilute 1 volume of the *Wash Buffer Concentrate (25X)* with 24 volumes of deionized water (e.g., 50 mL may be diluted up to 1.25 liters, 100 mL may be diluted up to 2.5 liters). Label as Working Wash Buffer.

Store both the concentrate and the Working Wash Buffer in the refrigerator. The diluted buffer should be used within 14 days.

ASSAY METHOD: PROCEDURE AND CALCULATIONS

Be sure to read the *Procedural Notes/Lab Quality Control* section before carrying out the assay.

Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

Note: A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)
2. Add 100 µL of the *Standard Diluent Buffer* to zero wells. Well(s) reserved for chromogen blank should be left empty.
3. Add 100 µL of standards, controls, and diluted samples (dilute samples at least 1:2 with *Standard Diluent Buffer*) to the appropriate microtiter wells. Tap gently on side of plate to thoroughly mix. (See **REAGENT PREPARATION AND STORAGE**, Section B.)

4. Cover wells with *plate cover* and incubate for **2 hours at room temperature.**
5. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
6. Pipette 100 µL of *Hu Aggregated Aβ Biotin Conjugate* solution into each well except the chromogen blank(s). Tap gently on the side of the plate to mix.
7. Cover wells with *plate cover* and incubate for **1 hour at room temperature.**
8. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
9. Add 100 µL Streptavidin-HRP Working Solution to each well except the chromogen blank(s). (Prepare the working dilution as described in **REAGENT PREPARATION AND STORAGE**, Section C.)
10. Cover wells with the *plate cover* and incubate for **30 minutes at room temperature**.
11. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
12. Add 100 µL of *Stabilized Chromogen* to each well. The liquid in the wells will begin to turn blue.
13. Incubate for **30 minutes at room temperature and in the dark**. ***Please Note*: Do not cover the plate with aluminum foil or metalized mylar**. The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density

(O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceed the limits of the instrument. The O.D. values at 450 nm can only be read after the *Stop Solution* has been added to each well. If using a reader that records only to 2.0 O.D., stopping the assay after 20 to 25 minutes is suggested.

14. Add 100 µL of *Stop Solution* to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
15. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 µL each of *Stabilized Chromogen* and *Stop Solution*. Read the plate within 2 hours after adding the *Stop Solution.*
16. Plot the absorbance of the standards against the standard concentration. (Optimally, the background absorbance may be subtracted from *all* data points, including standards, unknowns and controls, prior to plotting.) Draw the best smooth curve through these points to construct the standard curve. If using curve fitting software, the four parameter algorithm provides the best curve fit.
17. Read the aggregated Aβ concentrations for unknown samples and controls from the standard curve plotted in step 16. **Multiply value(s) obtained for sample(s) by the appropriate dilution factor to correct for the dilution with *Standard Diluent Buffer*.** (Samples producing signals higher than the highest standard (5.7 ng/mL) should be further diluted in *Standard Diluent Buffer* and reanalyzed, multiplying the concentration by the appropriate dilution factor.)

TYPICAL DATA

The following data were obtained for the various standards over the range of 0 to 5.7 ng/mL aggregated Aβ.

Standard Aggregated Aβ (ng/mL)	Optical Density (450 nm)
5.7	3.09
2.9	2.22
1.4	1.46
0.71	0.573
0.36	0.368
0.18	0.257
0.09	0.171
0	0.089

LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the 5.7 ng/mL standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute samples >5.7 ng/mL with *Standard Diluent Buffer;* reanalyze these and multiply results by the appropriate dilution factor.

The influence of various extraction buffers has not been thoroughly investigated. The rate of degradation of native aggregated Aβ in various matrices has not been investigated. Although aggregated Aβ degradation in the tissue extraction buffer described in this protocol has not been seen to date, the possibility of this occurrence cannot be excluded.

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PERFORMANCE CHARACTERISTICS

SENSITIVITY

The analytical sensitivity of this assay is <0.01 ng/mL of aggregated Aβ. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.

PRECISION

1. Intra-Assay Precision

Samples of known aggregated Aβ concentrations were assayed in replicates of 16 to determine precision within an assay.

	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	2.84	1.48	0.32
SD	0.19	0.09	0.03
%CV	6.68	6.29	9.26

SD = Standard Deviation
CV = Coefficient of Variation

2. Inter-Assay Precision

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

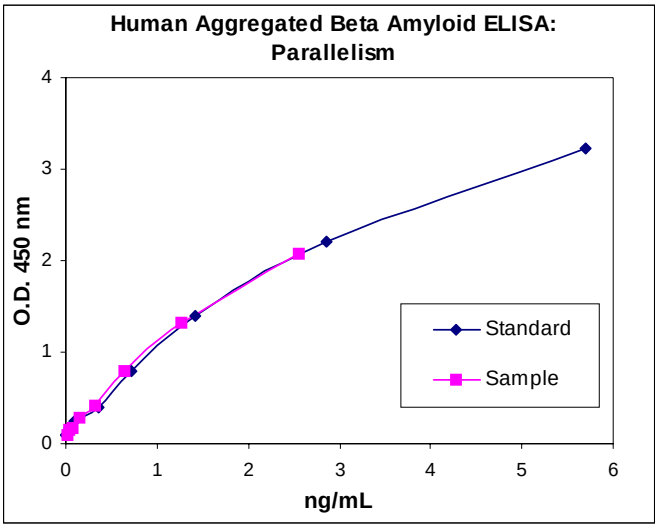
	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	2.82	1.36	0.33
SD	0.16	0.12	0.03
%CV	5.74	8.99	7.61

SD = Standard Deviation
CV = Coefficient of Variation

PARALLELISM

Aggregated Aβ peptide was serially diluted in *Standard Diluent Buffer*. The optical density of each dilution was plotted against the aggregated Aβ standard curve. Parallelism demonstrated by the figure below indicates that the standard accurately reflects aggregated Aβ content in a sample.

Figure 1



LINEARITY OF DILUTION

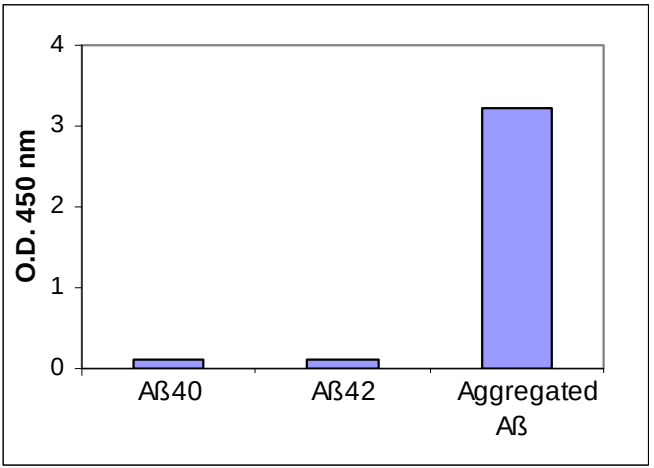
Aggregated Aβ was diluted in *Standard Diluent Buffer* over the range of the assay and measured. Linear regression analysis of sample values versus the expected concentrations yielded a correlation coefficient of 0.99.

	Cell Lysate		
Dilution	**Measured (ng/mL)**	**Expected (ng/mL)**	**% Expected**
Neat	2.55	2.55	100
1/2	1.35	1.28	106
1/4	0.737	0.638	115
1/8	0.344	0.319	108
1/16	0.214	0.159	134
1/32	0.088	0.079	110

SPECIFICITY

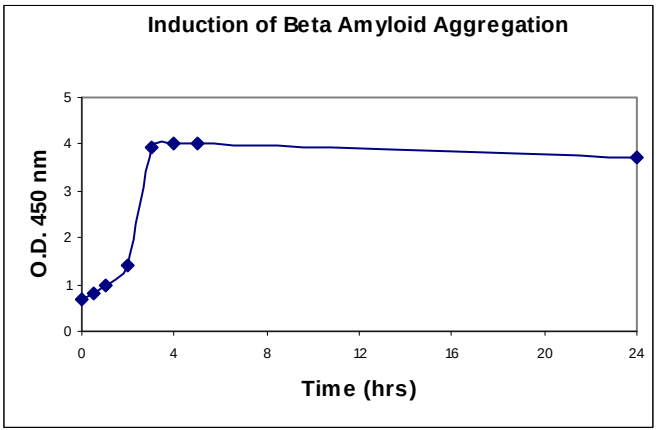
Aβ40 peptide, Aβ42 peptide, and aggregated Aβ were analyzed in the Human Aggregated Beta Amyloid ELISA. The data confirm the specificity of the assay for the aggregated form of Aβ, but not the non-aggregated Aβ40 or Aβ42 peptide.

Figure 2



Human Aβ40 peptides were treated with 100% HFIP at 1 mg/mL for 1 hour then dried over a gentle stream of nitrogen gas. When all of the HFIP solution was evaporated, the peptide was resuspended with water and incubated at 37°C. Samples were collected over a 24 hour period and analyzed in the Human Aggregated Beta Amyloid ELISA. The data demonstrate that Aβ aggregation appeared to reach maximal level at 4 hours post-reconstitution.

Figure 3



REFERENCES

1. Lambert, M.P., et al. (1998) Diffusible, non-fibrillar ligands derived from Abeta 1-42 are potent central nervous system neurotoxins. Proc. Nat'l. Acad. Sci. 95(11):6448-6453.
2. Gong, Y., et al. (2003) Alzheimer's disease-affected brain: Presence of oligomeric Aβ ligands (ADDLs) suggests a molecular basis for reversible memory loss. Proc. Nat'l. Acad. Sci. 100 (18):10417-10422.
3. Barghorn, S., et al. (2005) Globular amyloid β-peptide1-42-a homogeneous and stable neuropathological protein in Alzheimer's disease. J. Neurochem. 95(3):834-847.
4. Patel, N.S., et al. (2005) Inflammatory cytokine levels correlate with amyloid load in transgenic mouse models of Alzheimer's disease. J. Neuroinflammation, 2:9.
5. Mehlhorn, G., et al. (2000) Induction of cytokines in glial cells surrounding cortical beta-amyloid plaques in transgenic Tg2576 mice with Alzheimer pathology. Int. J. Dev. Neurosci. 18:423-431.
6. Apelt, J. and R. Schiebs (2001) Beta-amyloid-induced glial expression of both pro- and anti-inflammatory cytokines in cerebral cortex of aged and transgenic Tg2576 mice with Alzheimer plaque pathology. Brain Res. 891:21-30.
7. Abbas, N., et al. (2002) Up-regulation of the inflammatory cytokines IFN-gamma and IL-12 and down-regulation of IL-4 in cerebral cortex regions of APP(SWE) transgenic mice. J. Neuroimmunol. 126:50-57.
8. Gitter, B.D., et al. (2000) Regulation of cytokine secretion and amyloid precursor protein processing by proinflammatory amyloid beta. Ann. N.Y. Acad. 917:154-164.

9. Rah, J.C., et al. (2001) Effect of carboxyl-terminal fragment of Alzheimer's amyloid precursor protein and amyloid beta-peptide on the production of cytokines and nitric oxide in glial cells. FASEB J. 15:1463-1465.
10. Paris, D., et al. (2002) Pro-inflammatory effect of freshly solubilized beta-amyloid peptides in the brain. Prostaglandins Other Lipid Mediat. 70:1-12.
11. Giovannini, M.G., et al. (2002) Beta-amyloid-induced inflammation and cholinergic hypofunction in the rat brain *in vivo*: involvement of the p38 pathway. Neurobiol. Dis. 11:257-274.
12. Quadros, A., et al. (2003) Increased TNFalpha production and Cox-2 activity in organotypic brain slice cultures from APPsw transgenic mice. Neurosci. Lett. 353:66-68.
13. Rosales-Corral, S., et al. (2004) Kinetics of neuroin-flammationoxidative stress correlation in rat brain following the injection of fibrillar amyloid-beta onto the hippocampus *in vivo*. J. Neuroimmunol. 150:20-28.
14. Wegiel, J., et al. (2001) The role of microgial cells and astrocytes in fibrillar plaque evolution in transgenic APP (SW) mice. Neurobiol. Aging 22:49-61.
15. Vehmas, A.K., et al. (2003) Immune reactive cells in senile plaques and cognitive decline in Alzheimer's disease. Neurobiol. Aging 23:321-331.
16. Del Bo, R., et al. (1995) Reciprocal control of inflammatory cytokines, IL-1, IL-6, and beta-amyloid production in cultures. Neurosci. Lett. 188:70-74.

17. Griffin, W.S., et al. (1998) Glial-neuronal interactions in Alzheimer's disease: the potential of a "cytokine cycle" in disease progression. Brain Pathol. 8:65-72.
18. McGeer, P.L., et al. (2001) Inflammation, autotoxicity and Alzheimer's disease. Neurobiol. Aging 22:799-809.
19. Ma, Q.-L., et al. (2005) Antibodies against β-amyloid reduce Aβ oligomers, glycogen synthase kinase 3-β activation and phosphorylation *in vivo* and *in vitro*. J. Neurosci. Res. 83:374-384.
20. Zhao, L., et al. (2005) Role of p21-activated kinase pathway defect in the cognitive deficits of Alzheimer disease. Nat. Neurosci. 9:234-242.
21. Calon, F., et al. (2004) Docosahexaenoic acid protects from dendritic pathology in an Alzheimer's disease mouse model. Neuron 43:633-645.

Explanation of symbols

Symbol	Description	Symbol	Description
	Catalogue Number		Batch code
	Research Use Only		*In vitro* diagnostic medical device
	Use by		Temperature limitation
	Manufacturer		European Community authorised representative
[-]	Without, does not contain	[+]	With, contains
	Protect from light		Consult accompanying documents
	Directs the user to consult instructions for use (IFU), accompanying the product.		

Human Aggregated Beta Amyloid Assay Summary