



β -Amyloid (1-42) ELISA Kit

Cat. No. 99-0064

Size: 96 Tests

For Research Use Only

Lot No.

The β -Amyloid (1-42) ELISA Kit is an enzyme-linked immunosorbent sandwich assay for quantitative detection of human β -Amyloid (1-42) in cell culture lysates, and supernatants. For Research Use Only.

INTRODUCTION

Alzheimer's Disease (AD) is characterized by the presence of extracellular plaques and intracellular neurofibrillary tangles (NFTs) in the brain. The major protein component of these plaques is beta amyloid peptide (A β), a 40 to 43 amino acid peptide cleaved from amyloid precursor protein by β -secretase and a putative γ -secretase. Increased release of the 'longer forms' of A β peptide, A β 42 or A β 43, have a greater tendency to aggregate than A β 40, occurs in individuals expressing certain genetic mutations, expressing certain 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 which leads to the abnormal deposition of A β and the associated neurotoxicity in the brains of affected individuals. It is reported that a distinct A β peptide, A β N3(pE), is deposited in senile plaques in a dominant and differential manner as compared with the standard A β N1 peptide. Thus, it is very useful for AD research to assay A β 40, A β 42, A β 43, and A β N3 (pE), respectively.

REAGENTS AND MATERIALS PROVIDED

- 1x 96 well-plate precoated with Rabbit anti-Human β -Amyloid (38-42)
- 1 vial of HRP conjugated Rabbit anti-Human β -Amyloid (11-28) (0.4 ml) (30X)
- 1 vial of EIA buffer (30 ml)
- 2 vial of Standard Human β -Amyloid (1-40) (0.5 ml)
- 1 vial of Stop solution (12ml)
- 1 vial of TMB Chromogen solution (15 ml)
- 1 vial of 40x Wash buffer (50ml)
- 1 vial of Solution for Labeled Antibody (12 ml)

MATERIALS REQUIRED BUT NOT PROVIDED

- 5 ml and 10 ml graduated pipettes, beakers, flasks, and cylinders
- 10 μ l to 1,000 μ l adjustable single channel micropipettes with disposable tips
- 50 μ l to 300 μ l adjustable multichannel micropipette, disposable tips, and reservoir
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)

STORAGE

2° - 8°C

PRINCIPLE OF TEST

This kit is a solid phase sandwich ELISA using 2 kinds of high specific antibodies. Tetra methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The strength of coloring is in proportion to the quantities of human β -Amyloid (1-42).

ASSAY RANGE

13 pg/ml - 800 pg/ml

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PREPARATION OF REAGENTS

1. Preparation of wash buffer

Wash buffer is a concentrated (40X) buffer. Adjust the temperature of wash buffer to room temperature, and mix it gently and completely before use. Dilute 25ml of wash buffer with 975ml of distilled water and mix it. Store this prepared wash buffer at 2°-8°C and use within 2 weeks.

2. Solution of labeled antibody

Put 10.5ml of Solution for Labeled Antibody into the vial of HRP conjugated Rabbit anti-Human β -Amyloid and place it at room temperature for 5 minutes. Then mix it gently and completely. This step should be done just before the application of labeled antibody.

3. Preparation of standard

Put 0.5 ml of distilled water into the vial of standard and mix it gently and completely. This solution is 1600 pg/ml human β -Amyloid.

4. Dilution of standard

Prepare 8 tubes for dilution of standard. Put 230ul each of EIA buffer into tube.

Put 230ul of Standard solution into tube-1 and mix it gently. Then, put 230ul of tube-1 mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 800 pg/ml and 13 pg/ml, and tube-8 is the blank as 0 pg/ml as follow.

Tube-1	800 pg/ml
Tube-2	400 pg/ml
Tube-3	200 pg/ml
Tube-4	100 pg/ml
Tube-5	50 pg/ml
Tube-6	25 pg/ml
Tube-7	13 pg/ml
Tube-8	0 pg/ml

5. Dilution of samples

Samples may be diluted with EIA buffer if necessary.

PROCEDURE

1. Determine wells for blank, test sample and diluted standard. Then, put 100ul each of blank (tube-8), test sample and dilutions of standard (tubes-1-7) into the appropriate wells.
2. Incubate the plate for 1 hour at 37°C after covering it with plate lid.
3. Wash each well of the plate vigorously with wash buffer using washing bottle. Fill each well with wash buffer, and let it sit for 15~30 seconds. Remove wash buffer completely from the plate. Repeat 3 times. Remove the remaining liquid from all wells completely by snapping the plate onto paper towel.
4. Pipette 100ul of labeled antibody solution into the wells of test samples, diluted standard and test sample blank.
5. Incubate the plate for 30 minutes at 4°C after covering it with plate lid.
6. Wash the wells in plate.
7. Pipette 100ul TMB buffer into wells.
8. Incubate the plate for 30 minutes at room temperature in the dark. The liquid will turn blue by the addition of TMB buffer.
9. Pipette 100ul of Stop solution into the wells. Mix the liquid by tapping the side of the plate. The liquid will turn yellow by the addition of Stop solution.
10. Remove any drop of water on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the plate reader and conduct measurement at 450nm. The measurement should be done within 30minutes after the addition of Stop solution.

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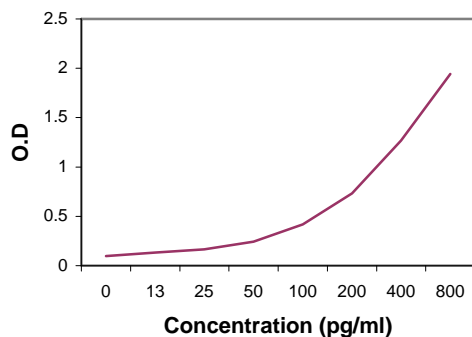
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CALCULATION OF RESULTS

Subtract the absorbance of test sample blank from all data, including standards and unknown samples before plotting. Plot the subtracted absorbance of the standards against the standard concentration. Draw the best smooth curve through these points to construct the standard curve. Read the concentration for unknown samples from the standard curve.

Representative standard curve for β -Amyloid (1-42)



PERFORMANCE CHARACTERISTICS

(1) Titer Assay

Specimen	Titer (X)	Measurement Value (pg/ml)	Theoretical Value (pg/ml)	%
10% FBS added RPMI-1640	×2	165.8	200	82.9
	×4	83.7	100	83.7
	×8	43.9	50	87.8
	×16	18.8	25	75.2
Serum (Std added)	×4	35.8	200	17.9
	×8	20.7	100	20.7
	×16	13.4	50	26.8
	×32	8.5	25	34.0
Cerebrospinal fluids (Std added)	×2	93.9	200	47.0
	×4	55.5	100	55.5
	×8	29.9	50	59.8
	×16	15.0	25	60.0

(2) Specificity

A β (1-40)	$\leq 0.1\%$
A β (1-43)	$\leq 0.1\%$

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(3) Inter - Assay

Measurement Value (pg/ml)	SD value	CV value (%)	N
488.4	6.89	1.41	8
167.8	4.60	2.74	8
39.4	2.74	6.95	8

(4) Intra - Assay

Measurement Value (pg/ml)	SD value	CV value (%)	N
437.3	44.88	10.26	3
145.4	19.48	13.40	3
35.2	4.71	13.38	3

RELATED PRODUCTS

Specificity	Clone/PAD	Catalog Number
Ms x Amyloid β -peptide	AMY-33	13-0100
Rb x Amyloid β -peptide	Polyclonal	71-5800
Ms x Amyloid β -Precursor Protein 695	LN27	13-0200
Ms x Amyloid β -Precursor Protein	CT695	51-2700
β -Amyloid (1-40) ELISA Kit	N/A	99-0063

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