

Titer Assay

Specimen	Titer (X)	Measurement Value (pg/ml)	Theoretical value (pg/ml)	%
10% FCS added RPMI-1640	2	158.83	209.54	75.8
	4	101.96	104.67	97.4
	8	53.54	51.65	103.7
	16	28.48	27.32	104.2
Human Plasma (EDTA) (x10)	8	17.99	50.00	36.0
	16	19.09	25.00	76.3
	32	20.39	19.96	102.2
	64	17.08	15.96	107.0
Human Cerebrospinal fluids (x10)	2	155.04	225.04	68.9
	4	107.23	113.95	94.1
	8	58.96	57.73	102.1

Added Recovery Assay

Specimen	Theoretical value (pg/ml)	Measurement Value (pg/ml)	%
10% FCS added RPMI-1640	206.68	181.43	87.8
	106.68	97.14	91.1
	56.68	50.63	89.3
Human Plasma (EDTA)	100.00	69.03	69.0
	50.00	28.36	56.7
	25.00	12.80	51.2
Human Cerebrospinal fluids .	206.24	208.73	101.2
	106.24	103.62	97.5
	56.24	52.88	94.0

kit PROFILE

Date received: _____ **Cat #** 200-110-A42 **Lot #** _____ **Exp.** _____

Date kit opened _____ **Technician:** _____

Date used: _____ **# Strips used** _ **# Remaining** _____

Remarks _____

Human Amyloid- β 1-42

ELISA Kit Cat. # 200-110-A42

For Quantitative Determination of Amyloid-Beta
In Human Serum, plasma, culture medium or other biological fluids

For In Vitro Research Use Only



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Human Amyloid Beta 1-42 ELISA KIT Cat. # 200-110-A42

Kit Components	96 tests
Rabbit Anti-Human Ab 1-42 IgG#1 coated strip plate (96 wells), # 200111	1 plate
Purified recombinant Human Ab1-42 Standard Stock (1600 pg/ml; see vial for exact concn (Powder) reconstitute in 0.5 ml distilled water; # 200112	2 Vials
EIA Buffer , # 200113 (30 ml)	1 bottle
Anti-human Ab1-42-HRP conjugate Stock , (0.4 ml), 30X stock # 200114	1 vial
Antibody Conjugate Diluent 12 ml # 200115	1 bottle
Wash Buffer (40X) Dilute 1:40 before use (50 mls) #200110-WB	1 bottle
TMB Substrate Solution (15 ml) # 200110TM	1 bottle
Stop Solution (1N H2SO4), (12 ml) # 200111	1 bottle
Instruction Manual, 200-110-A42	1

ADI's Amyloid Beta 1-42 (AB 1-42) ELISA is designed to measure human AB 1-42 in human serum, plasma, cerebrospinal fluids, cell culture media or the extract from brain tissue. Most normal serum or plasma samples have AB 1-42 that are below the detection levels.. The kit also detects recombinant or native human AB 1-42. For in vitro research use only.

Introduction

Alzheimer's Disease (AD) is a neurodegenerative disorder characterized by progressive loss of memory and cognition in the elderly. A number of genes have been linked in the initiation and development of AD. One of the most important and initial step involves proteolytic cleavage of amyloid precursor protein (APP, chromosome 21) releasing short 40, 42 & 43 aa peptides (beta amyloids 1-40, 1-42, and 1-43). Polymerization of beta-amyloid (Ab) and subsequent neuronal deposit (amyloid) leads to the degeneration of neurons involved in memory and cognition. Mutations in the APP gene cause some forms of familial AD (FAD) by releasing an increased amounts of b-amyloid. The AD Ab deposits also contain anti-chymotrypsin (ACT), and Apolipoprotein (Apo-E) that may promote Ab polymerization. Although, Ab deposits or plaques are central to neuropathogenesis and neurodegeneration, it is not clear how it affect neuronal functions. An early onset of FAD has been linked to some 30 mutations in two related genes, Presenilins-1 (PS-1 on chromosome 14; 467 aa) and Presenilins-2 (PS-2 on chromosome 1; 448 aa). PS-1/2 has been co-localized in subcellular sites involved in cell cycle regulation and mitosis (the nuclear membrane, interphase kinetochore, etc).

PERFORMANCE CHARACTERISTICS

1. Detection Limit - Based on sixteen replicate determinations of the zero standard, the minimum AB1-42 concentration detectable using this assay <4 pg/ml. The sensitivity for this kit was determined using the guidelines under the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols. (National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.

2. PRECISION

Inter-assay precision: Three samples were run in 23 independent assays. The samples showed good intra-assay precision (3-5 % CV). The actual values were: mean 474.31 pg/ml (SD 15.7 pg/ml); 110.3 pg/ml (SD 4.8) and 27.5 pg/ml, SD 1.7 pg/ml).

Intra-assay precision: Three samples were run as replicates in 23 tests using the ELISA kit. The samples showed good intra-assay precision (7-9 % CV). The actual values were: mean 462.4 pg/ml, SD 24.8 pg/ml; 107.5pg/ml (SD 5.5) and 27.0 pg/ml, SD 1.6 pg/ml).

3. LINEARITY

A sample (with original AB 1-42 concentration of 443.5 was diluted (1:2, 1:4, 1:8, 1:16, 1:32, and 1:64) and AB1-42 values determined. The samples showed excellent mean recoveries of about 98%, respectively (range 89-109%).

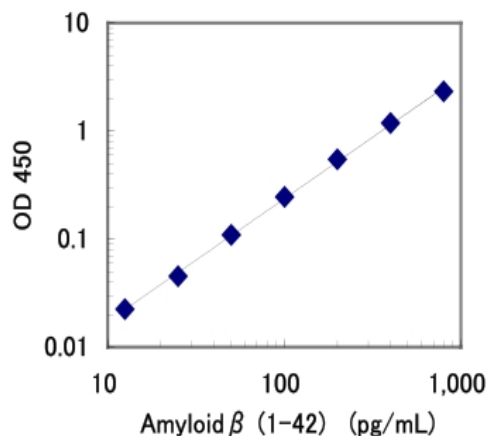
5. SPECIFICITY

Human AB 1-42	100%
Human AB 1-40	<0.1%
Human AB 1-43	<0.1%
Human AB 17-40 (P3 form)	<0.1%
Rat/Mouse AB1-40	<0.1%
Rat/Mouse AB1-42	71%

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Average A _{450nm}	Calculated concn.
A1, A2	Std. A (800 pg/ml)	2.479	
B1, B2	Std. B (400 pg/ml)	1.323	
C1, C2	Std. C (200 pg/ml)	0.688	
D1, D2	Std. D (100 pg/ml)	0.385	
E1, E2	Std. E (50 pg/ml)	0.249	
F1, F2	Std. F (25 pg/ml)	0.185	
G1, G2	Std. G (12.5 pg/ml)	0.162	
H1, H2	Std blank (0 pg/ml)	0.140	
A3, A4	Sample 1	0.395	101.0 pg/ml

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical std. assay curve (do not use this for calculating sample values)

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the blank from all stds and sample wells. Draw the standard curve on log-log graph paper by plotting net absorbance values of standards against appropriate AB 1-42 concentrations. Read off AB 1-42 concentrations of the control and patient samples.

PRINCIPLE OF THE TEST

Human AB 1-42 ELISA kit is based on binding of human AB 1-42 from standards or samples to a antibodies AB1-42 coated on the plate and anti AB 1-42-HRP conjugate. Higher concentrations of AB 1-42 in the sample result in increased binding of anti AB 1-42-enzyme (HRP) to the antibody coated plate. After a washing step, chromogenic substrate (TMB) is added and colors (blue) developed. The enzymatic reaction (color) is directly proportional to the amount of AB 1-42 present in the sample. Adding stopping solution terminates the reaction (blue color turns yellow). Absorbance is then measured using an ELISA reader at 450 nm. and the concentration of AB 1-42 in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (50-200 μ l) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plates Reader.

PRECAUTIONS

ADI's AB 1-42 ELISA kit is intended for *in vitro research* use only. The reagents contain cetylpyridinium chloride as preservative of conjugate and sodium azide in the standards. Appropriate care should be taken when disposing solutions. The stds./controls sera may contain human serum that has been shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

REAGENTS PREPARATION FOR THE ASSAY

- 1. Wash buffer Dilution:** Dilute stock (40X) wash buffer 1:40 with water (50 ml stock and 1950 mls of water; mix it thoroughly). It can be kept at 4oC for 2-4 weeks.
- 2. Preparation of HRP Labeled antibody -** Labeled antibody is a supplied as 30X solution (#2 vial). Dilute this 1:30 with "Conjugate diluent" (#5 vial), according to required quantity into a disposable test tube. Use this resulting solution as Labeled antibody. Example) In case you use one strip (8 well), the required quantity of Labeled antibody is 800 μ L. Prepare 900 ul of 1x solution (Dilute 30 μ L, 30X conjugate and 870 μ L of conjugate diluent). Prepare 10 ml for a full plate (333 ul 30X stock and 9.67 ml diluent). Note: Prepare 1X labeled antibody only in required amounts for use in a single test. Do not store diluted conjugate.

2. **Preparation Human AB 1-42 Standards:** Reconstitute **stock AB 1-42** standards in **0.5 ml water (stock concn 1600 pg/ml)**. Perform a 1:2 serial dilution with ELISA buffer # AB142-3 as follows:

	ELISA Buffer #3	Dilution	Final Concn	Std Name
225 ul stock 1600 pg/ml (as prepared above)	225 ul	1:2	800 pg/ml	A
225 ul of Std A	225 ul	1:4	400 pg/ml	B
225 ul of Std B	225 ul	1:8	200 pg/ml	C
225 ul of Std C	225 ul	1:16	100 pg/ml	D
225 ul of Std D	225 ul	1:32	50 pg/ml	E
225 ul of Std E	225 ul	1:64	25 pg/ml	F
225 ul of Std F	225 ul	1:128	12.5 pg/ml	G
Blank	225 ul	none	0 pg/ml	0 (blank)

When preparing serial dilution, make sure that the buffer and standards are mixed be gentle vortexing before taking aliquots for the next dilution. A total of 200 ul will be used for each run (100 ul used in duplicate). Prepare standards fresh and do not store for more than a few hours at 4oC. Reconstituted stock can be frozen at -20oC to -80oC for later use.

DILUTION OF SAMPLES

Samples containing >800 pg/ml AB 1-42 should be diluted with the EIA buffer. The results obtained should be multiplied by the appropriate dilution factor. If samples are too dilute, i.e. It may be necessary to test the samples at several dilution to determine optimum dilution. below the detection level, it may be necessary to prepare more concentrated cell lysate.

If fetal calf serum (FCS) etc. is contained in samples of culture supernatant, A β (1-42)-like in FCS may be measured. We recommend you to take the negative control. Serum or plasma may become below detection sensitivity in this kit due to very few concentration of A β (1-42) in these samples.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^oC until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. Due to the susceptibility of the some assay components, it is recommended that the entire kit is used immediately after reconstitution of the components or the components used within a few days.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

Human Amyloid- β 1-42 ELISA Procedure

Total Assay Time – Overnight

Allow **all** reagents to reach room temperature; arrange and label required # of strips. Dilute wash buffers, antibody conjugate, standard stock, and prepare Stds A-G water.

Step 1 Use EIA buffer (100 ul) **as blank** into 1 well. Pipet **100 ul of standards A-G and samples** in duplicate into appropriate wells.

Mix gently. Cover the plate and incubate at **4oC overnight (~12-16 h)**

Step 2 **Wash 5-times** with diluted wash buffer (300 ul/well/wash) if washing manually. Remove traces of liquid by tapping it over paper towels between each wash.

Step 3 Pipet **100 ul of diluted HRP conjugate**. Mix gently. Cover the plate and incubate at **4oC for 60 minutes**

Step 4 **Wash 7 times** as in Step 2. Remove traces of liquid by tapping the plate over clean paper towels.

Step 5 Add **100 ul TMB substrate, mix gently and incubate at room temp for 30 min.** blue color develops in standards and positive samples.

Step 6 Add **100 ul stop solution** into each well and mix gently. (Blue color turns yellow). Measure absorbance at 450nm within 30 minutes. Calculate unknown values using standard curve (see details at page 4).

Notes:

1. Test samples should be measured soon after the collection. In case of the storage of test samples, they should be stored under frozen conditions and do not repeat freeze/thaw cycles. Thaw the test samples at low temperature and mix them completely before measurement.
2. Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
3. Test samples should be diluted with "4, EIA buffer" , if the need arises.