Human Aß42 ELISA Kit

For the quantitative determination of human Aß42 concentrations in tissue homogenate, cell culture supernatant and other biological fluid.

Catalogue Number: EL10057
96 tests

FOR LABORATORY RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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INTENDED USE

This human Aß42 ELISA kit is to be used for the *in vitro* quantitative determination of human Aß42 concentrations in cell culture supernatant and other biological fluids. This kit is intended for LABORATORY RESEARCH USE ONLY.

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease that affects about 6% senior people worldwide. It is characterized by the formation of extracellular amyloid plaques, the accumulation of neurofibrillary tangles in neurons, and loss of neurons in some area of the brain. Amyloid beta peptide 42 (Aß42) has a tendency toward forming beta pleated structure and precipitation. It constituents the initial and key component of the insoluble amyloid fibril in the plaque. Aß42, like other amyloid peptides, is formed by the sequential cleavage of amyloid precursor protein by beta-secretase and gamma-secretase. Aß40, Aß42 and Aß43 are different only at the few C-end amino acids. Aß42 is believed to contribute to the pathogenesis of AD. One hypothetical mechanism is that the deposition of amyloid fibril onto the brain tissue results in AD. Another hypothesis is that the neurotoxicity of Aß42 oligomer is the cause of the disease.

Study suggested that lower than normal Aß42/ Aß40 ratio in cerebrospinal fluid could predicate development of AD. In blood and cerebrospinal fluid, the concentration of Aß40 is about 10 times higher than that of Aß42.

Hyper-phosphorylated Tau protein forms neurofibrillary tangles inside neurons. Study found that Aß42 level is lower and phosphorylated Tau protein level is higher in the cerebrospinal fluid of AD patients comparing with normal control and other dementia conditions.

This Aß42 ELISA kit can be used for measuring Aß42 in tissue homogenate, cell culture supernatant, and other biological fluids. The kit is for research use only, therefore, should not be used in any diagnostic or therapeutic procedures.

PRINCIPLE OF THE ASSAY

This enzyme-linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific for human Aß42. When standards or samples are added to the appropriate microtiter plate wells, human Aß42 in the standards or samples will be immobilized by the pre-coated antibody during incubation. Then, a biotin-conjugated antibody preparation specific for human Aß42 is added to each well and incubated. The biotin labelled antibody attaches to the wells by binding to human Aß42. After plate washing, other proteins, components and unattached biotin labelled antibody is removed. After that, avidin-horseradish peroxidase (HRP) conjugate is added to each well. Avidin has a very high affinity for biotin, thus, it links the tracer (HRP) sturdily to the

biotin labelled antibody. The wells are thoroughly washed to remove all unbound avidin-HRP conjugate and a TMB (3,3',5,5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only wells that contain human Aß42 will exhibit a change in colour. The extent of colour change is proportional to the quantity of human Aß42 presented in the standards/samples. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wave length of 450 nm ± 2 nm.

According to the testing system, the provided standard is diluted (2-fold) with the appropriate diluent and assayed at the same time as the samples. This allows the operator to produce a curve of Optical Density (O.D.) versus human Aß42 concentration (pg/mL) in standards. The concentration of human Aß42 in the samples is then determined by comparing the O.D. of the samples to the standard curve and multiplying with sample dilution factor.

In order to measure the concentration of human Aß42 in tissue samples, a Tissue Homogenization Buffer is included in the kit for sample processing.

REAGENTS PROVIDED
All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

	96 tests

1. **HUMAN Aß42 MICROTITER PLATE** (Part EL57-1) ***96 wells***
   Pre-coated with anti-human Aß42 monoclonal antibody

2. **BIOTIN CONJUGATE** (Part EL57-2) ***6 mL***
   Anti-human Aß42 monoclonal antibody conjugated to biotin

3. **AVIDIN-HRP CONJUGATE** (Part EL57-3) ***12 mL***
   Avidin conjugated to horseradish peroxidase

4. **HUMAN Aß42 STANDARD** (Part EL57-4) ***2 vials***
   Recombinant human Aß42 (2000pg/vial) in a buffered protein base with preservative, lyophilized.

5. **STANDARD/SAMPLE DILUENT** (Part EL57-5) ***60 mL***
   Add 2mM 4-(2-aminoethyl)-benzenesulfonyl fluoride (AEBSF) before use.

6. **TISSUE HOMOGENIZATION BUFFER** (Part EL57-6) ***30 mL***
   For extraction of amyloid peptides from tissue samples.

7. **WASH BUFFER (20X)** (Part 30005) ***60 mL***
   20-fold concentrated solution of buffered surfactant.

8. **SUBSTRATE A** (Part EL57-7) ***11 mL***
   Buffered solution with urea hydrogen peroxidase.

9. **SUBSTRATE B** (Part 30007) ***11 mL***
   Buffered solution with TMB.

10. **STOP SOLUTION** (Part 30008) ***14 mL***
    2N Sulphuric Acid (H_2SO_4). Caution: Caustic Material!

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips (10-100μL and 50-200μL) for running the assay.
2. Pipettes: 1 mL, 5 mL, and 10 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Eppendorf tubes and racks.
5. 4-(2-aminoethyl)-benzenesulfonyl fluoride (AEBSF) or protease inhibitor cocktail containing AEBSF.
6. Micro tissue grinder
7. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
8. Microtiter plate reader (450 nm ± 2nm)
9. Automatic microtiter plate washer or squirt bottle.
10. Sodium hypochlorite solution, 5.25% (household liquid bleach).
11. Deionized or distilled water.
12. Plastic plate cover.
13. Disposable gloves.
14. Absorbent paper.

PRECAUTIONS

1. Thimerosal interferes with the assay. Please use other preservative for reagent preparation.
2. Tissue Homogenization Buffer contains guanidine hydrochloride which is a harmful substance. Wear googles and protective gloves when handling the buffer. Contact with the buffer can cause severe damage to the eyes and inflammation to skin. Spills should be cleaned with large amount of water.
3. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
4. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
5. Do not use kit components beyond their expiration date.
6. Use only deionized or distilled water to dilute reagents.
7. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
8. Use fresh disposable pipette tips for each transfer to avoid contamination.
9. Do not mix acid and sodium hypochlorite solutions.
10. All biological samples should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn and good laboratory practices should be followed during the assay procedure.
11. All samples should be disposed of in a manner that will inactivate viruses.
    Solid Wastes: Autoclave for 60 minutes at 121°C.
    Liquid Wastes: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate viruses before disposal.

10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form which must be dissolved by warming to 37°C prior to use.
12. Tissue Homogenization Buffer may appear opaque. The buffer should be vortexed before use.

SAMPLE PREPARATION

COLLECTION, PROCESSING, STORAGE AND DILUTION:

Cell Culture Supernatant: Dilute sample 1:1 with Cell Culture Medium containing 1mM AEBSF, or 1mM AEBSF in protease inhibitor cocktail.

- Samples must be assayed immediately, or stored at -20°C or lower temperature. When performing the assay, bring samples to room temperature.

Tissue Samples:

- Collect tissue samples and weigh the tissue on balance.
- Determine the amount of TISSUE HOMOGENIZATION BUFFER needed by the formula: Volume (μL) of TISSUE HOMOGENIZATION BUFFER = 8 x tissue weight (mg).
- Put the tissue into an Eppendorf tube and leave on ice.
- Vortex the TISSUE HOMOGENIZATION BUFFER and add 100-200μL into the Eppendorf tube containing the tissue.
- Grind the tissue thoroughly with micro tissue grinder while gradually add the rest of the TISSUE HOMOGENIZATION BUFFER.
- Mix the homogenate on shaker at room temperature for 3 hours.
- Use micro-centrifuge to remove particles at 16,000g and 4ºC for 20 minutes.
- Samples must be assayed immediately, or stored at -20°C or lower temperature.
- Before assay starts, dilute the sample with cold STANDARD/SAMPLE DILEUNT containing 1mM AEBSF. The range of dilution may need to be adjusted according to sample types to ensure that the OD readings of the diluted samples are within standard curve range. Dilute about 1:200 for double transgenic (APPswe/PS1dE9) mouse AD brain sample. This Aβ42 ELISA, targeting human Aβ 42, should also be able to detect Aβ42 in human tissue.
- If the SAMPLE DILUTION is ≤1:10, the STANDARD DILEUNT should be added with the same proportion of TISSUE HOMOGENIZATION BUFFER, to generate a more relevant standard curve.

Other Biological Fluid:

- Use STANDARD/SAMPLE DILEUNT or a buffer similar to the samples to dilute standards and samples. Add AEBSF to 2mM before use.
- Blood samples contain factors that interfere with the assay. Removing these factors may be necessary to obtain consistent results.

PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as below.

1. **Standard/Sample Diluent:** Add 1mM pure AEBSF, or 1mM AEBSF in protease inhibitor cocktail, to appropriate STANDARD/SAMPLE DILUENT (Ref to SAMPLE PREPARATION) before use.

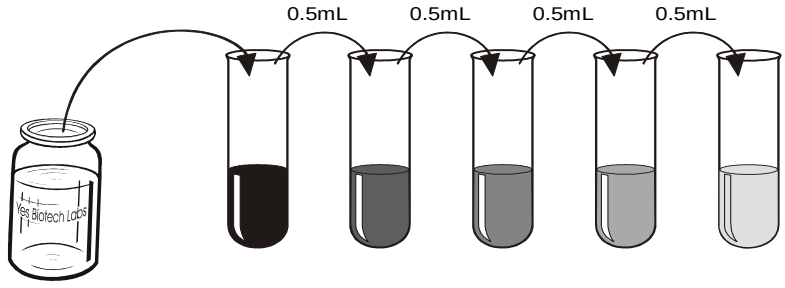
2. **Wash Buffer (1X):** Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water.

3. **Human Aß42 Standard:**

   Two vials of Aß42 standard are included in the kit. The standard is a fusion protein containing the complete amino acid sequence of Aß42. The amount of the fusion protein in standard has been adjusted to reflect the molar concentration of unmodified Aß42 at 2ng/vial.

   a) Reconstitute the human Aß42 Standard with 4mL of appropriate Standard Diluent. This reconstitution produces a stock solution of 500pg/mL. Allow solution to sit for at least 15 minutes with gentle agitation prior to making dilutions. Use within one hour of reconstitution. Aliquot the stock and store frozen at -20°C (short term) or -70°C (long term) if repeated use is expected.

   b) Use the above stock solution to produce a 2-fold serial dilution within the range of this assay (15.6pg/mL to 500pg/mL) as illustrated. Add 0.5mL of the Standard Diluent to each test tube. Between each test tube transfer, be sure to mix contents thoroughly. The undiluted human Aß42 standard will serve as the high standard (500pg/mL) and the Standard Diluent will serve as the zero-standard (0pg/mL).



500pg/mL 250pg/mL 125pg/mL 62.5pg/mL 31.25pg/mL 15.6pg/mL

ASSAY PROCEDURE

1. Prepare Wash Buffer (1X), human Aß42 Standards and Samples before starting assay procedure (Refer to Sample Preparation and Preparation of Reagents). *It is recommended that the table and diagram provided be used as a reference for adding Standards or Samples to the Microtiter Plate.*

Wells	Contents	**Wells**	Contents
1A, 1B	Standard 1 - **0pg/mL** (S1)	**2A, 2B**	Standard 5 – **125pg/mL** (S5)
1C, 1D	Standard 2 – **15.6pg/mL** (S2)	**2C, 2D**	Standard 6 – **250pg/mL** (S6)
1E, 1F	Standard 3 – **31.25pg/mL** (S3)	**2E, 2F**	Standard 7 – **500pg/mL** (S7)
1G, 1H	Standard 4 – **62.5pg/mL** (S4)	**2G,2H**	Human Aß42 **samples**

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	S1	S5	2	6	10	14	18	22	26	30	34	38
B	S1	S5	2	6	10	14	18	22	26	30	34	38
C	S2	S6	3	7	11	15	19	23	27	31	35	39
D	S2	S6	3	7	11	15	19	23	27	31	35	39
E	S3	S7	4	8	12	16	20	24	28	32	36	40
F	S3	S7	4	8	12	16	20	24	28	32	36	40
G	S4	1	5	9	13	17	21	25	29	33	37	41
H	S4	1	5	9	13	17	21	25	29	33	37	41

2. Add 100µL of Standards or Samples to the appropriate wells of anti-human Aß42 antibody pre-coated microtiter plate.

3. Add 50µL Anti-human Aβ42 Biotin conjugate to the wells. Mix 30-60 seconds on a plate shaker. Cover plate and incubate overnight at 4 °C

4. Wash the Microtiter Plate using one of the specified methods indicated below:

   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer (1X) then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note*: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

   Automated Washing: Aspirate all wells, then wash plates **FIVE times** using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350µL/well/wash (range: 350-400 µL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no

moisture appears. *It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes.*

5. Dispense 100µL of Avidin-HRP conjugate to each well. Cover and incubate on a plate shaker for 1 hour at room temperature.

6. Repeat wash procedure as described in Step 4.

7. Add 100µL Substrate Solution to each well. Cover and incubate for 15 minutes at room temperature.

8. Add 100µL Stop Solution to each well. Mix well.

9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 30 minutes.

CALCULATION RESULT

The standard curve is used to determine the amount of human Aß42 in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding human Aß42 concentration (pg/mL) on the horizontal (X) axis.

1. First, calculate the mean O.D value for each standard and sample. All O.D. values are subtracted by the value of the zero-standard (0pg/mL) before result interpretation. Construct the standard curve using graph paper or statistical software.

2. To determine the amount of human Aß42 in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding human Aß42 concentration. If samples generate values higher than the highest standard, dilute the samples with the appropriate Standard Diluent and repeat the assay, the concentration read from the standard curve must be multiplied by the dilution factor.

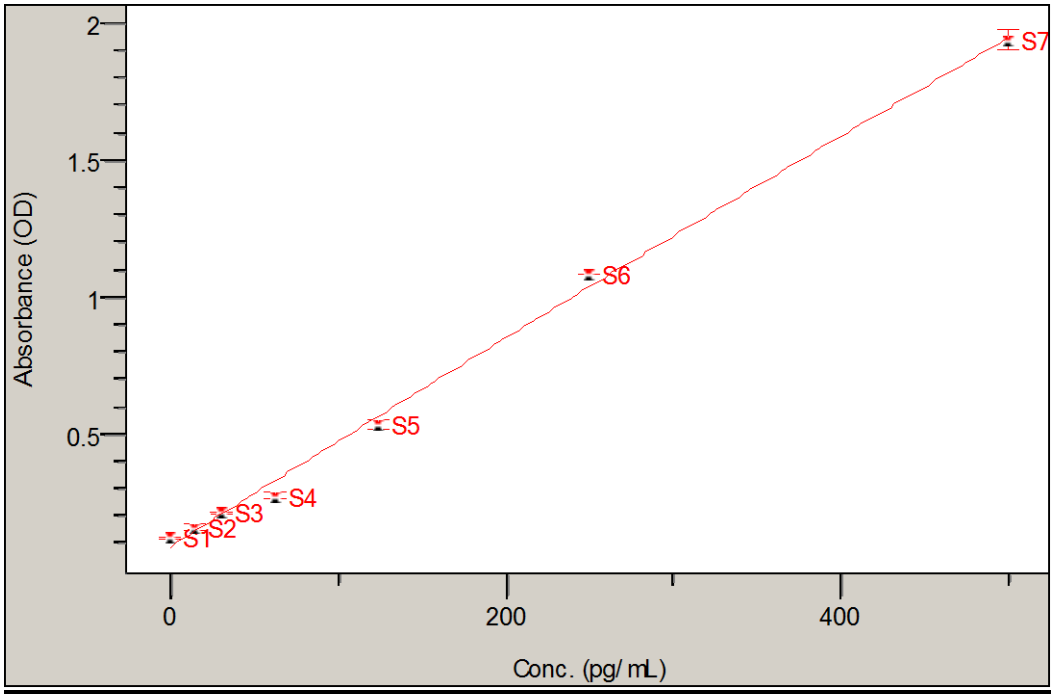
TYPICAL DATA

Results of a typical standard run of a human Aß42 ELISA are shown below. Any variation in standard diluent, operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of illustration only, and should not be used to calculate user results.

EXAMPLE ONE

The following data was obtained using STANDARD/SAMPLE DILUENT.

Standard (pg/mL)	Mean O.D. (450 nm)	%CV	Zero Standard Subtracted (Std.)-(S1)
0	0.126	4.49	0
15.6	0.165	6.00	0.039
31.25	0.216	0.68	0.09
62.5	0.278	4.84	0.152
125	0.539	3.15	0.413
250	1.088	0.62	0.962
500	1.942	1.81	1.816



PERFORMANCE CHARACTERISTICS

1. INTRA-ASSAY PRECISION

To determine within-run precision, three different samples of known concentration added were assayed by using 16 replicates in 1 assay.

Sample	1	2	3
N	16	16	16
Mean (pg/ml)	42.8	216.1	463.9
Coefficient of Variation (%)	6.81	6.67	5.69

2. INTER-ASSAY PRECISION

To determine between-run precision, three different samples of known concentration were measured by using replicates on 8 different assays.

Sample	1	2	3
N	8	8	8
Mean (pg/mL)	50.1	229.9	468.5
Coefficient of Variation (%)	7.7	7.1	3.6

3. Antigenic Specificity

The following substances were tested and found to have no cross-reactivity: human Aß40 (100ng/mL), human Aß43 (10ng/mL), α-Synuclein (100ng/mL).

4. RECOVERY

The recovery of human Aß42 added to cell culture medium averaged 97.3%.

4. SENSITIVITY

The minimum detectable dose of human Aß42 was determined by adding two standard deviations to the mean optical density value of 16 zero standard replicates and calculating the corresponding concentration from the standard curve. The minimum detectable dose of human Aß42 calculated from calibrate diluted standard curve was <6.54pg/mL.

REFERENCES

Andreasen N et al., Cerebrospinal fluid beta-amyloid (1-42) in Alzheimer disease: differences between early- and late-onset Alzheimer disease and stability during the course of disease. 1999. Arch Neurol. 56(6): 673-80

Hansson N et al., Prediction of Alzheimer's disease using the CSF Abeta42/Abeta40 ratio in patients with mild cognitive impairment. 2007. Dement Geriatr Cogn Disord., 23(5): 316-20

Olsson F et al., Characterization of intermediate steps in amyloid beta (Aβ) production under near-native conditions. 2014. J. Biol. Chem. 289(3): 1540-50

Shankar GM et al., Amyloid b-protein dimers isolated directly from Alzheimer brains impair synaptic plasticity and memory. 2008. Nat. Med. 14(8): 837–42.