## Human Amyloid-β 1-40 ELISA Procedure Summary
## 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** | Pipet **100 ul of standards A-G and samples** in duplicate into appropriate wells. Use EIA buffer (100 ul) as blank (see page 3 for details). |
| | Mix gently. Cover the plate and incubate at **4oC overnight (~12-16 h)** |
| **Step 2** | **Wash 7-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. Wash 5 times if automated ELISA washer is used. |
| **Step 3** | Pipet **100 ul** of **diluted HRP conjugate**. Mix gently. Cover the plate and incubate at **4oC for 60 minutes** |
| **Step 4** | Wash 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.** A blue color develops. |
| **Step 4** | 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). |

## KIT PROFILE

**Date received**: **Cat #** 200-100-A40 **Lot #** ___ **Exp.** _____

**Date kit opened** _____ **Technician:** _____

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

**Remarks** _____

_____

_____



*Instruction Manual No. 200-100-A40*

# Human Amyloid-β 1–40

## ELISA Kit Cat. # 200-100-A40

### For Quantitative Determination of Amyloid-Beta In Human Serum

*For In Vitro Research Use Only*

ALPHA DIAGNOSTIC INTERNATIONAL

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**DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.**

**Human Amyloid Beta 1-40 ELISA KIT Cat. # 200-100-A40**

| Kit Components | 96 tests |
|---|---|
| Mouse Anti-Human Ab1-40 antibody coated strip plate (96 wells), **# A B 4 0 - 1** | 1 plate |
| Purified recombinant Human **Ab1-40 Standard Stock** (2000 pg/ml; see vial for exact concn (Powder) reconstitute in 1.0 ml distilled water; **# AB40-2** | 2 Vials |
| EIA Buffer, # **AB40-3** (30 ml) | 1 bottle |
| Rabbit Anti-human Ab1-40-HRP conjugate Stock, **# AB40-4** (0.4 ml), **30X stock** | 1 vial |
| Ab1-40-HRP conjugate Diluent **# AB40-5** (12 ml) | 1 bottle |
| TMB Substrate cat # **AB40-6** (15 ml) | 1 bottle |
| Stop Solution (1N H2SO4), **# AB40-7 (12 ml)** | 1 bottle |
| Wash Buffer (40X) Dilute 1:40 before use **Cat # AB40-8 (50 mls)** | 1 bottle |
| Instruction Manual, 2 0 0 - 1 0 0 - A 4 0 | 1 |

## 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).

ADI's AB 1-40 ELISA is designed to measure human AB 1-40 in human serum, plasma, cerebrospinal fluids, or cell culture media.

Antibodies and related peptides can also be purchased individually from our site.



**PERFORMANCE CHARACTERISTICS**

1. **Detection Limit** - Based on sixteen replicate determinations of the zero standard, the minimum AB1-40 concentration detectable using this assay <3-5 pg/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

**2. PRECISION**

*Inter-assay precision:* Three samples were run in 8 independent assays. The samples showed good intra-assay precision (3-8 % CV). The actual values were: mean 408.9 pg/ml (SD 15.7 pg/ml); 78.6 pg/ml (SD 6.8) and 32.5 pg/ml, SD 1.7 pg/ml).

*Intra-assay precision:* Three samples were run as replicates and tested using the ELISA kit. The samples showed good intra-assay precision (7-9 % CV). The actual values were: mean 381.4 pg/ml, SD 37.8 pg/ml; 84.5pg/ml (SD 6.5) and 35.0 pg/ml, SD 3.0 pg/ml).

**3. LINEARITY**

A sample (with original AB 1-40 concentration of 443.5 was diluted (1:2, 1:4, 1:8, 1:16, 1:32, and 1:64) and AB1-40 values determined. The samples showed excellent mean recoveries of about 98%, respectively (range 89-109%).

**5. SPECIFICITY**

There are several variants of amyloid-beta produced by cultured cells. The antibodies employed in the kit are directed against 35-40 (capture antibody) and 1-6 aa of Amyloid beta 1-40 (tracer antibody). Therefore, human AB1-40 variants differing in amino acids at the N-terminus are not detected. Antibody crossreactivity with some AB 1-40 variants was determined:

| | |
|---|---|
| Human AB 1-40 | 100% |
| Human AB 1-42 | <0.1% |
| Human AB 17-40 (P3 form) | <0.1% |
| Rat/Mouse AB1-40 | <16% |
| Rat/Mouse AB1-42 | <1.5% |

Human β 1-43: DAEF**R**HDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIAT

Sequence of 1-6 aa in AB1-40 is the same in human, pig, monkey, sheep, g. pig, canine, rabbit, bovine, frog and chicken. Since this kit uses 1-6 aa antibody as tracer, it is expected that this kit should detect AB1-40 in these species. Rat/Mouse 1-40 have "G" at position 5 and it is not detected very well in this kit.

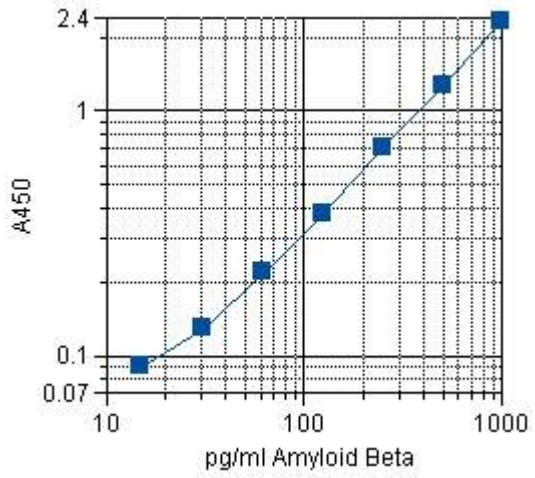
**ELISA Kits available from ADI:**

| | |
|---|---|
| Human Amyloid 1-40 ELISA Kit | # 200-100-A40 |
| Human Amyloid 1-42ELISA Kit | # 200-110-A42 |

## WORKSHEET OF TYPICAL ASSAY

| Wells | Stds/samples | Av. $A_{450nm}$ | Calculated concn. |
|---|---|---|---|
| A1, A2 | **Std. A** (1000 pg/ml) | 2.417 | |
| B1, B2 | **Std. B** (500 pg/ml) | 1.328 | |
| C1, C2 | **Std. C** (250 pg/ml) | 0.70 | |
| D1, D2 | **Std. D** (125 pg/ml) | 0.390 | |
| E1, E2 | **Std. E** (62.5 pg/ml) | 0.235 | |
| F1, F2 | **Std. F** (31.3 pg/ml) | 0.130 | |
| G1, G2 | **Std. G** (15.6 pg/ml) | 0.095 | |
| H1, H2 | **Std blank** (0 pg/ml) | 0.040 | |
| | | | |
| A3, A4 | **Sample 1** | 0.710 | 251 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 1-40 concentrations. Read off AB 1-40 concentrations of the control and patient samples.



### PRINCIPLE OF THE TEST

Human AB 1-40 ELISA kit is based on binding of human AB 1-40 from standards or samples to a rabbit polyclonal AB1-40 (35-40 aa) coated on the plate and anti AB 1-40 (1-6 aa)-HRP conjugate. Higher concentrations of AB 1-40 in the sample result in increased binding of anti AB 1-40-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 1-40 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 1-40 in samples and control is read off the standard curve.

### MATERIALS AND EQUIPMENT REQUIRED

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

### PRECAUTIONS

ADI's AB 1-40 ELISA kit is intended for *in vitro research* use only. The reagents contain cetylpyidinium 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 (**25 ml stock and 975 mls of water; mix it thoroughly**. It can be kept at 4oC for 2-4 weeks. Wash buffer 1X= PBS pH 7.5 and 0.05% Tween-20.

2. ***Preparation Human AB 1-40 Standards***: Reconstitute **stock** AB 1-40 standards in **1.0 ml water (stock concn 2000 pg/ml).** Perform a 1:2 serial dilution with ELISA buffer # AB140-3 as follows:

| | ELISA Buffer #3 | Dilution | Final Concn | Std Name |
|---|---|---|---|---|
| 225 ul stock 2000 pg/ml (as prepared above) | 225 ul | 1:2 | 1000 pg/ml | A |
| 225 ul of Std A | 225 ul | 1:4 | 500 pg/ml | B |
| 225 ul of Std B | 225 ul | 1:8 | 250 pg/ml | C |
| 225 ul of Std C | 225 ul | 1:16 | 125 pg/ml | D |
| 225 ul of Std D | 225 ul | 1:32 | 62.5 pg/ml | E |
| 225 ul of Std E | 225 ul | 1:64 | 31.3 pg/ml | F |
| 225 ul of Std F | 225 ul | 1:128 | 15.6 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.

3. **#AB40-4 antibody-HRP Conjugate** (30X)- dilute 1:30 before use with conjugate diluent # AB40-5. You will need 100 ul/well or 800 ul/strip of 8-wells or 10 mls or 1-full plate.

-Take 30 ul of HRP-conjugate and 870 ul of diluent (for 1-strip)

-Take 300 ul of HRP-conjugate and 8.7 ml of diluent (for 12-strips)

Prepare the conjugate in the required volume and do not keep diluted stock after the assay. The conjugate diluent must be at room temperature before its use.



**STORAGE AND STABILITY**

The microtiter well plate and all other reagents, if unopened, are stable at 2-8$^{O}$C 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.

**DILUTION OF SAMPLES**

Samples containing >1000 pg/ml AB 1-40 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.

**Performance Characteristics**

Dilutions of samples

Known concentrations of AB1-40 (230 pg/ml) were serially diluted (1:12; 1:4, 1:8, 1:16; 1:32; 1:64) in 10% FCS/RPMI1640 culture medium, Human serum, Human Plasma (EDTA), and Human cerebrospinal fluid (CSF) and AB140 concentration measured in the assay. Recovery of Ab140 was 80-99%.