



## ELISA Kit

Catalog #    **KHB3481 (96 tests)**  
                  **KHB3482 (192 tests)**

# *Human* **A $\beta$ 40**

[www.invitrogen.com](http://www.invitrogen.com)

Invitrogen Corporation

542 Flynn Road, Camarillo, CA 93012

Tel: 800-955-6288

E-mail: [techsupport@invitrogen.com](mailto:techsupport@invitrogen.com)



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## Contents and Storage

**Storage** Store at 2 to 8°C.

### Contents

Reagents Provided	96 Test Kit	192 Test Kit
<i>Hu Aβ40</i> Standard. Lyophilized synthetic peptide. Refer to vial label for quantity and reconstitution volume.	1 vial	1 vial
<i>Standard Diluent Buffer</i> . Contains 0.1% sodium azide; red dye*; 60 mL per bottle.	1 bottle	2 bottles
<i>Aβ Antibody Coated Wells</i> . 96 Well Plate. Plate pre-coated with mAb to NH <sub>2</sub> terminus of Aβ.	1 plate	2 plates
<i>Hu Aβ40 Detection Antibody</i> . Contains 0.1% sodium azide; blue dye*; 6 mL per bottle.	1 bottle	2 bottles
<i>Anti-Rabbit IgG HRP (100X)</i> . Contains 3.3 mM thymol; 0.125 mL per vial.	1 vial	2 vials
<i>HRP Diluent</i> . Contains 3.3 mM thymol; yellow dye*; 25 mL per bottle.	1 bottle	1 bottle
<i>Wash Buffer Concentrate (25X)</i> . 100 mL per bottle.	1 bottle	1 bottle
<i>Stabilized Chromogen, Tetramethylbenzidine (TMB)</i> . 25 mL per bottle.	1 bottle	1 bottle
<i>Stop Solution</i> . 25 mL per bottle.	1 bottle	1 bottle
<i>Plate Covers, adhesive strips</i> .	2	4
* In order to help our customers avoid any mistakes in pipetting the reagents, we provide colored <i>Standard Diluent Buffer</i> , <i>Detection Antibody</i> , and <i>HRP Diluent</i> to help monitor the addition of solution to the reaction well. 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.

**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.

# Introduction

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## Purpose

The Invitrogen Human A $\beta$ 40 ELISA is to be used for the quantitative determination of Hu A $\beta$ 40 in samples (e.g., tissue culture medium, tissue homogenate, cerebrospinal fluid (CSF), etc.). The assay will recognize both natural and synthetic forms of Hu A $\beta$ 40. The anti-human A $\beta$ 40 antibody used in this kit is capable of selectively detecting A $\beta$ 40 and not A $\beta$ 42/A $\beta$ 43 (1-3).

**For Research Use Only. CAUTION: Not for human or animal therapeutic or diagnostic use.**

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## Principle of the Method

The Invitrogen Hu A $\beta$ 40 kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for the NH<sub>2</sub>-terminus of Hu A $\beta$  has been coated onto the wells of the microtiter strips provided.

During the first incubation, standards of known Hu A $\beta$ 40 content, controls, and unknown samples are pipetted into the wells and co-incubated with a rabbit antibody specific for the COOH-terminus of the 1-40 A $\beta$  sequence. This COOH-terminal sequence is created upon cleavage of the analyzed precursor.

Bound rabbit antibody is detected by the use of a horseradish peroxidase-labeled anti-rabbit antibody. After washing, horseradish peroxidase-labeled anti-rabbit antibody (enzyme) is added. After a second incubation and washing to remove all the unbound enzyme, 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 Hu A $\beta$ 40 present in the original specimen.

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## Background Information

Beta amyloid peptide (A $\beta$ ), a 40 to 43 amino acid peptide cleaved from amyloid precursor protein by  $\beta$ -secretase (e.g., BACE) and a putative  $\gamma$  (gamma) secretase (3). Increased release of the 'longer forms' of A $\beta$  peptide, A $\beta$ 42 or A $\beta$ 43, which have a greater tendency to aggregate than A $\beta$ 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 $\beta$ 42/A $\beta$ 43 which leads to the abnormal deposition of A $\beta$  (4).

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## Methods

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### Materials Needed But Not Provided

- Distilled or deionized water
  - Standard Reconstitution Buffer [55 mM Sodium Bicarbonate Buffer (NaHCO<sub>3</sub>, ultrapure grade), pH 9.0]
  - 4-(2-aminoethyl)-benzenesulfonyl fluoride (AEBSF) or protease inhibitor cocktail containing AEBSF.
  - Microtiter plate reader (at or near 450 nm) with software
  - Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.)
  - Shaking platform (for low to moderate shaking) with plate
  - Calibrated adjustable precision pipettes
  - Glass or polypropylene tubes for diluting solutions
  - Absorbent paper towels
  - Calibrated beakers and graduated cylinders
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### Procedural Notes

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 collected in pyrogen/endotoxin-free tubes.
  4. When analyzing samples, add a protease inhibitor cocktail with AEBSF (a serine protease inhibitor) and prepare the standard dilutions using the same diluent as used with the biological samples. Serine proteases can rapidly degrade A $\beta$  peptides, thus using AEBSF (water soluble and less toxic than PMSF) at a 1 mM final concentration is very helpful. Keep samples on ice until ready to apply to plate.
  5. 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.
  6. Sample matrix has a dramatic impact on A $\beta$  recovery. To ensure accurate quantitation, the standard curves must be generated in the same diluent as the samples.
  7. Avoid use of thymol or thimerosal as sample preservatives. These agents inhibit measurement of A $\beta$  peptide.
  8. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
  9. It is recommended that all standards, controls and samples be run in duplicate.
  10. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
  11. **Do not mix or interchange different reagent lots from various kit lots.**
  12. Do not use reagents after the kit expiration date.
  13. Absorbances should be read immediately, but can be read up to 2 hours after assay completion. For best results, keep plate covered in the dark.
  14. In-house controls or kit controls, if provided, should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
  15. 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.
  16. Because Stabilized *Chromogen* is light sensitive, avoid prolonged exposure to light. Avoid contact between chromogen and metal, or color may develop.
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**Directions  
for Washing**

- **Incomplete washing will adversely affect the test outcome.** All washing must be performed with the *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 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 Buffer*. Let soak for 15 to 30 seconds, and then aspirate the liquid. Repeat as directed under Assay Procedure. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
  - Alternatively, the diluted *Wash Buffer* may be put into a squirt bottle. If a squirt bottle is used, flood the plate with the diluted *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, follow the washing instructions carefully.
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# Preparation of Reagents and Samples

## Preparing Standard Reconstitution Buffer

Dissolve 2.31 grams of sodium bicarbonate in 500 mL of deionized water. Add 2 N sodium hydroxide until pH is 9.0. Filter solution through a 0.2  $\mu\text{m}$  filter unit.

**Note:** This buffer is used to reconstitute the lyophilized standard. Do not use this buffer to dilute the standard or samples.

## Dilution of Standard

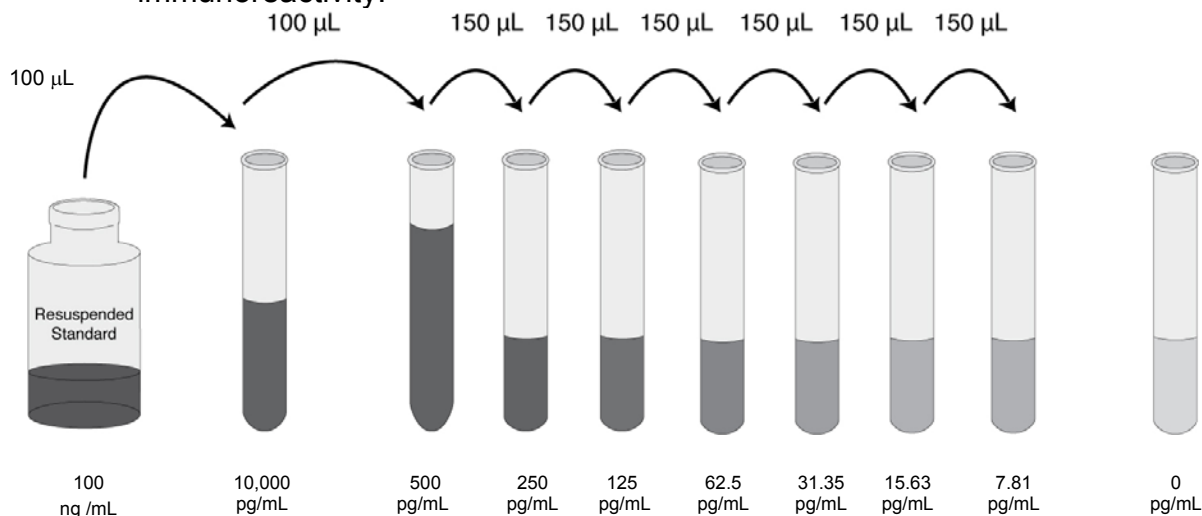
**Note:** Polypropylene tubes may be used for standard dilutions.

The Hu A $\beta$ 40 Standard is calibrated against highly purified Hu A $\beta$  where mass was corrected for peptide content by amino acid analysis.

1. Remove the *Hu A $\beta$ 40 Standard* vial from storage and let equilibrate to room temperature (RT).
2. Reconstitute standard to 100 ng/mL with *Standard Reconstitution Buffer* (55 mM sodium bicarbonate, pH 9.0). Refer to the standard vial label for instructions. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Briefly vortex prior to preparing standard curve.
3. Generation of the standard curve using the A $\beta$  peptide standard must be performed using the same composition of buffers used for the diluted experimental samples. For example, if brain extracts are diluted 1:10 with Standard Diluent Buffer, then the buffer used to dilute standards should be 90% Standard Diluent Buffer and 10% brain extraction buffer (including AEBSF at a final concentration of 1 mM).
4. Add 0.1 mL of the reconstituted standard to a tube containing 0.9 mL of the *Standard Diluent Buffer*. Label as 10,000 pg/mL Hu A $\beta$ 40. Mix.
5. Add 0.1 mL of the 10,000 pg/mL standard to a tube containing 1.9 mL *Standard Diluent Buffer*. Label as 500 pg/mL Hu A $\beta$ 40. Mix.
6. Add 0.15 mL of *Standard Diluent Buffer* to each of 6 tubes labeled 250, 125, 62.5, 31.25, 15.63, 7.81, and 0 pg/mL Hu A $\beta$ 40.
7. Make serial dilutions of the standard as described in the following dilution diagram. Mix thoroughly between steps.

## Note

Remaining reconstituted Hu A $\beta$ 40 standard may be stored in aliquots at  $-80^{\circ}\text{C}$  for up to 4 months. Standard can be frozen and thawed one time only without loss of immunoreactivity.





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**Preparing Secondary Antibody**

**Note: Prepare within 15 minutes of usage.** The *Anti-Rabbit IgG HRP (100X)* is in 50% glycerol, which is viscous. To ensure accurate dilution, allow *Anti-Rabbit IgG HRP (100X)* to reach room temperature. Gently mix. Pipette *Anti-Rabbit IgG HRP (100X)* slowly. Remove excess concentrate solution from pipette tip by gently wiping with clean absorbent paper.

1. Dilute 10  $\mu\text{L}$  of this 100X concentrated solution with 1 mL of *HRP Diluent* for each 8-well strip used in the assay. Label as Anti-Rabbit IgG HRP Working Solution.
2. Return the unused *Anti-Rabbit IgG HRP (100X)* to the refrigerator.

# of 8-Well Strips	Volume of Anti-Rabbit IgG HRP (100X)	Volume of Diluent
2	20 $\mu\text{L}$ solution	2 mL
4	40 $\mu\text{L}$ solution	4 mL
6	60 $\mu\text{L}$ solution	6 mL
8	80 $\mu\text{L}$ solution	8 mL
10	100 $\mu\text{L}$ solution	10 mL
12	120 $\mu\text{L}$ solution	12 mL

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**Dilution of Wash Buffer**

1. 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.
2. Store both the concentrate and the Working Wash Buffer in the refrigerator. The diluted buffer should be used within 14 days.

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**Sample Preparation**

Prepare one or more dilutions of each sample. These dilutions should be made in *Standard Diluent Buffer*, although the exact dilution must be determined empirically (e.g., 1:2 and 1:10 represent a reasonable range). This dilution must be performed because certain components in samples can interfere with the detection of the A $\beta$  peptides or to bring the levels of A $\beta$  within the range of this assay. AEBSF should be added to the diluted samples and the standards at a final concentration of 1 mM in order to prevent proteolysis of the A $\beta$  peptides.

Refer to  $\beta$ -Amyloid Application (page 13) for procedure for homogenization of human or transgenic mouse brains.

**Note:** Analysis of plasma samples may require pretreatment to disrupt interaction of A $\beta$  with masking proteins.

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## Assay Procedure

**Be sure to read the *Procedural Notes* 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 50  $\mu\text{L}$  of the *Standard Diluent Buffer* to the zero standard wells. Well(s) reserved for chromogen blank should be left empty.
3. Prepare samples and standards with appropriate diluents. Add 50  $\mu\text{L}$  of  $\text{A}\beta$  peptide standards, controls, and samples to each well except for the chromogen blank (s).
4. Add 50  $\mu\text{L}$  of *Hu A $\beta$ 40 Detection Antibody* solution to each well except for the chromogen blank(s).
5. Cover plate with *plate cover* and incubate for **3 hours at room temperature with shaking**.
6. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **Directions for Washing**.
7. Add 100  $\mu\text{L}$  Anti-Rabbit IgG HRP Working Solution to each well except the chromogen blank(s). See **Preparation of Reagents**.
8. Cover plate with the *plate cover* and incubate for **30 minutes at room temperature**.
9. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **Directions for Washing**.
10. Add 100  $\mu\text{L}$  of *Stabilized Chromogen* to each well. The liquid in the wells will begin to turn blue.
11. Incubate for **30 minutes at room temperature and in the dark**.  
**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 exceeds 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 15 to 20 minutes is suggested.
12. Add 100  $\mu\text{L}$  of *Stop Solution* to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
13. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100  $\mu\text{L}$  each of *Stabilized Chromogen* and *Stop Solution*. Read the plate within 30 minutes after adding the *Stop Solution*.
14. Use a curve fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit.
15. Read the concentrations for unknown samples and controls from the standard curve. **Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.** (Samples producing signals greater than that of the highest standard should be further diluted in *Standard Diluent Buffer* and reanalyzed, multiplying the concentration found by the appropriate dilution factor.)

**Typical Data  
(Example)**

The following data were obtained for the various standards over the range of 0 to 500 pg/mL Hu A $\beta$ 40.

Standard Hu A $\beta$ 40 pg/mL	Optical Density (450 nm)
500	3.887
250	2.434
125	0.946
62.5	0.386
31.25	0.194
15.63	0.128
7.81	0.101
0	0.075

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# Performance Characteristics

## Analytical Sensitivity

The minimum detectable dose of Hu A $\beta$ 40 is <6 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 64 times, and calculating the corresponding concentration.

## Precision

### 1. Intra-Assay Precision

Samples of known Hu A $\beta$ 40 concentration were assayed in replicates of 14 to determine precision within an assay.

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	49.5	129.7	305.7
SD	1.4	3.3	7.1
%CV	2.8	2.5	2.3
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 (pg/mL)	50.8	130.0	298.2
SD	2.0	4.5	12.1
%CV	3.9	3.5	4.1
SD = Standard Deviation CV = Coefficient of Variation			

## Linearity of Dilution

Human CSF containing 195 pg/mL of measured Hu A $\beta$ 40 was serially diluted in *Standard Diluent Buffer* over the range of the assay. RPMI containing 10% fetal calf serum was spiked with the native Hu A $\beta$ 40 from CSF to a level of 212 pg/mL, and then serially diluted in *Standard Diluent Buffer* over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

Dilution	Cerebrospinal Fluid			Cell Culture Supernatant		
	Measured (pg/mL)	Expected (pg/mL)	% Expected	Measured (pg/mL)	Expected (pg/mL)	% Expected
neat	195	195	-	212	212	-
1/2	81	98	82.7	100	106	94.3
1/4	43	49	87.7	49	53	92.5
1/8	24	24	100	28	27	104

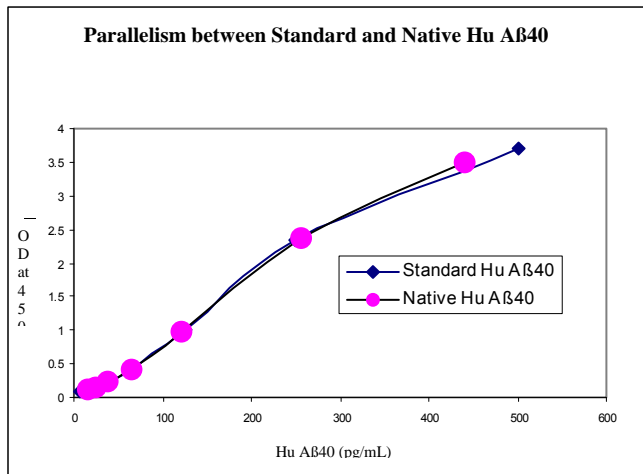
## Recovery

The recovery of native Hu A $\beta$ 40 added to human CSF averaged 87%. The recovery of native Hu A $\beta$ 40 added to tissue culture medium containing 10% fetal calf serum averaged 98%. The recovery of native Hu A $\beta$ 40 added to 25% tissue homogenate averaged 92%.

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**Parallelism**

Native A $\beta$ 40 was spiked into Standard Diluent Buffer and measured against the standard used in this kit. Parallelism between the two peptides is demonstrated by the figure below.



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**Antigenic Specificity**

Buffered solutions of a panel of substances of 50 ng/mL were assayed in the Hu A $\beta$ 40 kit. The following substances were tested and found to have no cross-reactivity: A $\beta$  [1-12], A $\beta$  [1-20], A $\beta$  [12-28], A $\beta$  [22-35], A $\beta$  [1-42], A $\beta$  [1-43], A $\beta$  [42-1],  $\alpha$ -synuclein (90 ng/mL), APP (20 ng/mL), and Tau (10 ng/mL). Rodent  $\beta$  Amyloid (1-40) showed approximately 0.5% cross reaction at 50 ng/mL.

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**High Hook Dose Effect**

Samples spiked with Hu A $\beta$ 40 peptide up to 1  $\mu$ g/mL gave responses higher than that obtained for the last standard point.

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**Limitations of the Procedure**

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

The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced, etc.) and the use of biological fluids in place of serum samples have not been thoroughly investigated. The rate of degradation of native Hu A $\beta$ 40 in various matrices has not been investigated. The immunoassay literature contains frequent references to aberrant signals seen with some sera, attributed to heterophilic antibodies. Though such samples have not been seen to date, the possibility of this occurrence cannot be excluded.

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# **$\beta$ -Amyloid Application: Procedure for homogenization of human or transgenic mouse brains**

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## **Prepare Solutions**

- A. 5 M guanidine HCl  
50 mM Tris HCl, pH 8.0
- B. Reaction Buffer BSAT-DPBS (Dulbecco's phosphate buffered saline with 5% BSA and 0.03% Tween-20, see formulation below) supplemented with 1x Protease Inhibitor Cocktail (e.g., Calbiochem catalog code 539131; contains AEBSF, aprotinin, E64, EDTA, and leupeptin).

### BSAT-DPBS Formulation

- 0.2 g/L KCl
- 0.2 g/L  $\text{KH}_2\text{PO}_4$
- 8.0 g/L NaCl
- 1.150 g/L  $\text{Na}_2\text{HPO}_4$
- 5% BSA
- 0.03% Tween-20
- q.s. to 1 L with ultrapure water and adjust the pH to 7.4.

## **Protocol**

1. Determine the wet mass of the mouse hemibrain (100 mg) or a human brain sample in an Eppendorf tube (Fisher K749520-0000).
2. Add 8x mass of cold 5 M guanidine HCl / 50 mM Tris HCl (Solution "A", above) to the tube by 50 - 100  $\mu\text{L}$  aliquots and grind thoroughly with a hand-held motor (Fisher K749540-0000) after each addition. (Optional: transfer the homogenate from above to a 1 mL Dounce homogenizer and homogenize thoroughly.)
3. Mix the homogenate at room temperature for 3 - 4 hours. The sample is stable and can be freeze-thawed many times at this stage.
4. Dilute the sample with cold Reaction Buffer (Solution "B", above). Centrifuge (microfuge or Sorvall) at 16,000 x g for 20 minutes at 4°C. This dilution factor requires adjustment depending on the quantity of A $\beta$  present and on inhibition of the standard curve development due to the presence of guanidine. Initial experiments indicate a dilution factor of 1:200 for human brain and 1:20 to 1:50 for transgenic mouse brains. The optimal dilution factor should be determined for each specific experimental determination. (Note: we have determined that the standard curve can withstand the presence of 0.1 M or less guanidine solution. Inclusion of guanidine at a concentration higher than 0.1 M will result in significant depression of the standard curve.)
5. Carefully decant the supernatant and store on ice until use with the  $\beta$ -Amyloid ELISA kit from Invitrogen.

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## **Alternative Procedure**

Homogenization can be performed with cold 4x volume of PBS supplemented with the 1x protease inhibitor cocktail, followed by the addition of a solution 8.2 M guanidine / 82 mM Tris HCl (pH 8.0) to yield a solution with 5 M final guanidine concentration.

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**References  
for  
Homogeni-  
zation  
Procedure**

1. Masliah, E., et al. (2001)  $\beta$ -amyloid peptides enhance  $\alpha$ -synuclein accumulation and neuronal deficits in a transgenic mouse model linking Alzheimer's disease and Parkinson's disease. *Proc. Nat'l. Acad. Sci.* 98(21):12245-12250.
  2. Johnson-Wood, K., et al. (1997) Amyloid precursor protein processing and A beta42 deposition in a transgenic mouse model of Alzheimer disease. *Proc. Nat'l. Acad. Sci.* 94:1550-1555.
  3. Chishti, M.A., et al. (2001) Early-onset amyloid deposition and cognitive defects in transgenic mice expressing a double mutant form of amyloid precursor protein 695. *J. Biol. Chem.* 276(24):21562-21570 (cites the use of KHB3442 and KHB3482 with brain homogenates).
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# Appendix

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## Troubleshooting Guide

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Elevated background

*Cause:* Insufficient washing and/or draining of wells after washing. Solution containing Anti-Rabbit IgG HRP can elevate the background if residual is left in the well.

*Solution:* Wash according to the protocol. Verify the function of automated plate washer. At the end of each washing step, invert plate on absorbent tissue on countertop and allow to completely drain, tapping forcefully if necessary to remove residual fluid.

*Cause:* Contamination of substrate solution with metal ions or oxidizing reagents.

*Solution:* Use distilled/deionized water for dilution of wash buffer and use plastic equipment. DO NOT COVER plate with foil.

*Cause:* Contamination of pipette, dispensing reservoir or substrate solution with Anti-Rabbit IgG HRP.

*Solution:* Do not use chromogen that appears blue prior to dispensing onto the plate. Obtain new vial of chromogen.

*Cause:* Incubation time is too long or incubation temperature is too high.

*Solution:* Reduce incubation time and/or temperature.

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Elevated sample/  
standard  
ODs

*Cause:* Incorrect dilution of standard stock solution; intermediary dilutions not followed correctly.

*Solution:* Follow the protocol instructions regarding the dilution of the standard.

*Cause:* Incorrect dilution of the Anti-Rabbit IgG HRP Working Solution.

*Solution:* Warm solution of Anti-Rabbit IgG HRP (100X) to room temperature, draw up slowly and wipe tip with laboratory wipe to remove excess. Dilute ONLY in HRP diluent provided.

*Cause:* Incubation times extended.

*Solution:* Follow incubation times outlined in protocol.

*Cause:* Incubations carried out at 37°C when RT is dictated.

*Solution:* Perform incubations at RT (= 25 ± 2°C) when instructed in the protocol.

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Poor  
standard  
curve

*Cause:* Improper preparation of standard stock solution.

*Solution:* Dilute lyophilized standard as directed by the vial label only with the standard diluent buffer or in a diluent that most closely matches the matrix of your sample.

*Cause:* Reagents (lyophilized standard, standard diluent buffer, etc.) from different kits, either different analyte or different lot number, were substituted.

*Solution:* NEVER substitute any components from another kit.

*Cause:* Errors in pipetting the standard or subsequent steps.

*Solution:* Always dispense into wells quickly and in the same order. Do not touch the pipette tip on the individual microwells when dispensing. Use calibrated pipettes and the appropriate tips for that device.

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Weak/no  
color  
develops

*Cause:* Reagents not at RT ( $25 \pm 2^\circ\text{C}$ ) at start of assay.

*Solution:* Allow ALL reagents to warm to RT prior to commencing assay.

*Cause:* Incorrect storage of components, e.g., not stored at 2 to  $8^\circ\text{C}$ .

*Solution:* Store all components exactly as directed in protocol and on labels.

*Cause:* Anti-Rabbit IgG HRP Working Solution made up longer than 15 minutes before use in assay.

*Solution:* Use the diluted Anti-Rabbit IgG HRP within 15 minutes of dilution.

*Cause:* TMB solution lost activity.

*Solution 1:* The TMB solution should be clear before it is dispensed into the wells of the microtiter plate. An intense aqua blue color indicates that the product is contaminated. Please contact Technical Support if this problem is noted. To avoid contamination, we recommend that the quantity required for an assay be dispensed into a disposable trough for pipetting. Any TMB solution left in the trough should be discarded.

*Solution 2:* Avoid contact of the TMB solution with items containing metal ions.

*Cause:* Attempt to measure analyte in a matrix for which the ELISA assay has not been optimized.

*Solution:* Please contact Technical Support for advice when using alternative sample types.

*Cause:* Wells have been scratched with pipette tip or washing tips.

*Solution:* Use caution when dispensing and aspirating into and out of microwells.

Poor  
Precision

*Cause:* Errors in pipetting the standards, samples or subsequent steps.

*Solution:* Always dispense into wells quickly and in the same order. Do not touch the pipette tip on the individual microwells when dispensing. Use calibrated pipettes and the appropriate tips for that device. Check for any leaks in the pipette tip.

*Cause:* Repetitive use of tips for several samples or different reagents.

*Solution:* Use fresh tips for each sample or reagent transfer.

*Cause:* Wells have been scratched with pipette tip or washing tips.

*Solution:* Use caution when dispensing and aspirating into and out of microwells.

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## Technical Support

### Contact Us



For more troubleshooting tips, information, or assistance, please call, email, or go online to [www.invitrogen.com/ELISA](http://www.invitrogen.com/ELISA).

#### USA:

Invitrogen Corporation  
542 Flynn Road  
Camarillo, CA 93012

Tel: 800-955-6288

E-mail: [techsupport@invitrogen.com](mailto:techsupport@invitrogen.com)

#### Europe:

Invitrogen Ltd  
Inchinnan Business Park  
3 Fountain Drive  
Paisley PA4 9RF, UK

Tel: +44 (0) 141 814 6100

Fax: +44 (0) 141 814 6117

E-mail: [eurotech@invitrogen.com](mailto:eurotech@invitrogen.com)

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
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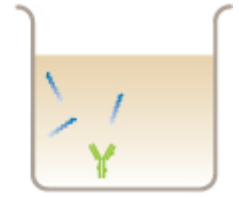
## Explanation of symbols

Symbol	Description	Symbol	Description
	Catalogue Number		Batch code
	Research Use Only		<i>In vitro</i> 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.		

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# Human A $\beta$ 40 Assay Summary

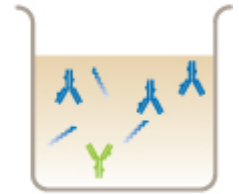
Add 50  $\mu$ L of Standards and Samples



Add 50  $\mu$ L of Detection Antibody  
Incubate for 3 hr (shaking) at RT



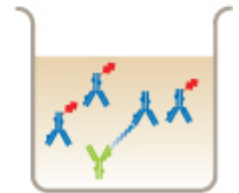
aspirate and wash 4x



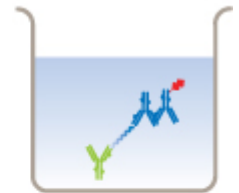
Incubate 100  $\mu$ L of HRP Anti-Rabbit Antibody  
for 30 minutes at RT



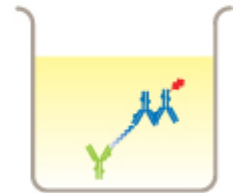
aspirate and wash 5x



Incubate 100  $\mu$ L of Stabilized Chromogen  
for 30 minutes at RT



Add 100  $\mu$ L of Stop Solution and read at 450 nm



Total time: 4 hr

