

For the quantitative determination of mouse A $\beta$ 42 concentrations in cell culture supernatants, serum, and plasma.

### INTRODUCTION

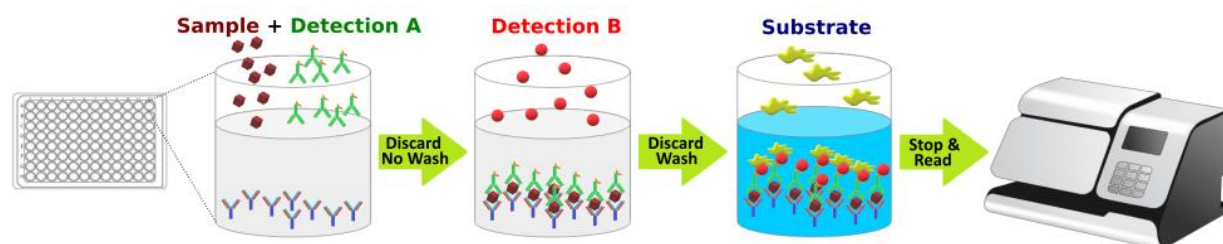
Beta-amyloid, a 40–43 amino acid peptide, is cleaved from amyloid precursor protein, by a putative gamma-secretase. Amyloid beta (1-40) and Amyloid beta (1-42) are two major C-terminal variants of the amyloid beta protein, constituting the majority of amyloid beta proteins. Both the amyloid beta (40) and amyloid beta (42) alloforms are major components of the extracellular plaques found in Alzheimer's disease brain tissue.

Tribio Fast Mouse A $\beta$ 42 ELISA is designed to quantitatively detect mouse A $\beta$ 42 levels in serum, plasma, and other biological samples. The kit features a novel proprietary one-step approach that combines sample incubation and detection, eliminating the need for complicated traditional multi-step procedures. This innovation makes the assay simple, easy to perform, accurate, and fast. The hands-on time can be completed within 2 hours, compared to the typical 4–5 hours required by conventional methods. The detection range spans from 78 to 5000 pg/mL. Measured mouse A $\beta$ 42 levels show a linear correlation with the standard curve generated using the kit standards. Therefore, this kit can be used to determine relative mass values for natural mouse A $\beta$ 42 protein.

### PRINCIPLE OF THE ASSAY

This assay employs our novel proprietary sandwich enzyme immunoassay techniques (Fig. 1). A monoclonal antibody specific for mouse A $\beta$ 42 is pre-coated onto a microplate. Standards or samples and a biotin conjugated detection antibody are pipetted into the wells and concurrently incubated to form a sandwich complex in one step. Simply aspirate each well without washing and directly add Streptavidin-HRP into the complex. Following a wash, an **ultra-sensitive TMB substrate solution** is added to the wells for color development. The color intensity is proportional to the amount of A $\beta$ 42 bound in the initial step. The intensity of the color is measured by plate reading at 450 nm.

Fig. 1



### KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Mouse A $\beta$ 42 Microplate	TBS3009A	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse A $\beta$ 42.	Return unused wells to the foil pouch. Reseal along the entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.
Mouse A $\beta$ 42 Standard	TBS3009B	50 $\mu$ l of Recombinant mouse A $\beta$ 42 (100 ng/mL).	Aliquot and store at -20 °C for up to 1 month in a manual defrost the freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS3009C	2.1 mL of biotin- mouse A $\beta$ 42 antibody.	May be stored for up to 3 months at 2-8 °C.
Detection B	TBS3009D	400 $\mu$ L of streptavidin HRP.	
Assay Diluent	TBS3009E	25 mL of a buffered protein base with preservatives.	
Wash Buffer	TBS3000W	12 mL of concentrated solution (10x)	
TMB Substrate	TBS3000T	12 mL of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6 mL of 2 N sulfuric acid.	

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

The kit contains sufficient materials to run an ELISA on one 96 well plate.

### PRECAUTIONS

Wear protective gloves, clothing, eyes, and face protection. Wash hands thoroughly after handling.

### REAGENT PREPARATION

**Bring all reagents to room temperature before use.**

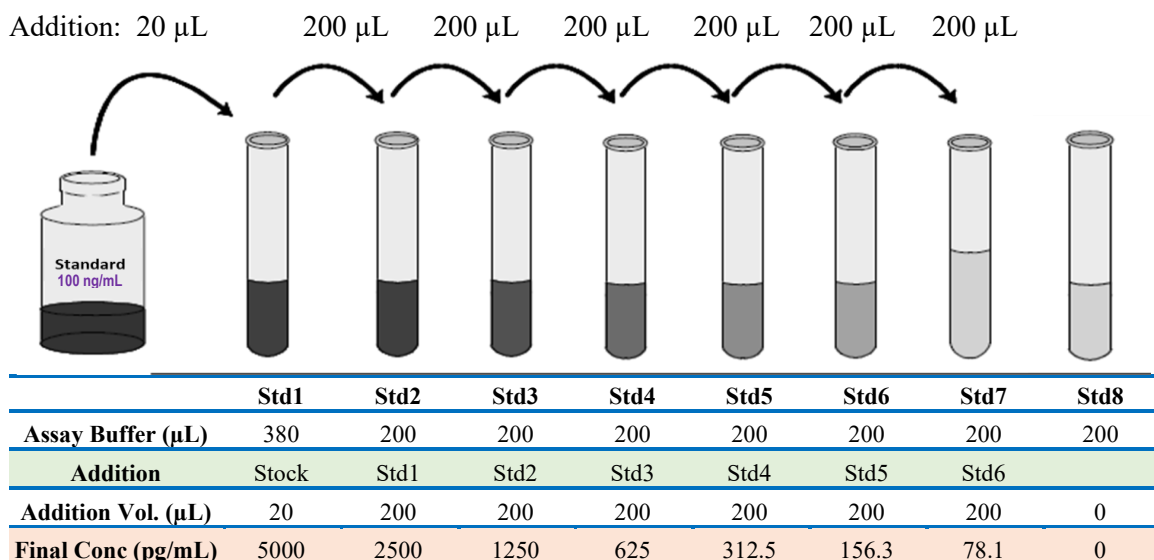
**Wash Buffer:** Add 12 mL of Wash Buffer Concentrate (10x) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved*).

**Detection B working solution preparation:** Add 240  $\mu$ L of **Detection B** streptavidin-HRP to 12 mL Assay Diluent (TBS3009E) to prepare Detection B working solution.

**Mouse A $\beta$ 42 Standard Preparation:** Label test tubes as #1 through #8. Pipet 380  $\mu$ L of 1x Assay Diluent into tube #1, and 200  $\mu$ L into tubes #2 to #8 **as diagram below**.

1. Add 20  $\mu$ L of the Mouse A $\beta$ 42 Standard stock solution (100 ng/mL) to tube #1 and mix.
2. Make 2x serial dilutions of the standard using the Tube#1(5000 pg/mL standard solution) from Tube #2 through #7 with sequential transfer of 200  $\mu$ L to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 5000, 2500, 1250, 625, 312.5, 156.3 and 78.1 pg/mL. Tube# 8 is Standard 8 (0 pg/mL).

**Fig.2 Diagram for Mouse A $\beta$ 42 standard preparation**



### ASSAY PROCEDURE

**Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.**

1. Add 80  $\mu$ L of standard, sample, or control per well.
2. Add 20  $\mu$ L of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT for 2 hours with shaking**.
3. Aspirate each well (no wash). Invert the plate and blot it against clean paper towels.
4. Add 100  $\mu$ L of **Detection B working solution** to each well. Incubate at **RT for 1 hour with shaking**.
5. Aspirate each well, and wash for 3 times by filling each well with 300  $\mu$ L Wash Buffer (*Complete removal of liquid at each step is essential to good performance*). After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100  $\mu$ L of **TMB Substrate** to each well. Incubate **at RT for 10-20 minutes with shaking** (*Protect from light*). The color becomes blue.
7. Add 50  $\mu$ L of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).
8. Determine the optical density of each well within 20 minutes, using a microplate reader at 450 nm. If wavelength

correction is available, set to 542 nm or 570 nm. If wavelength correction is not available, subtract readings at 542 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

### CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample subtract the average zero standard optical density (O.D.).

Create a standard curve using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

### TYPICAL DATA

This standard curve ( $R^2=1.000$ ) is provided for demonstration only. A standard curve should be generated for each set of samples assayed. Fig. 3 is an example of typical Data.

### SENSITIVITY

The minimum detectable dose (MDD) of mouses is typically 10 pg/mL.

The Intra-assay CV and the Inter-assay CV are <10%.

### SPECIFICITY

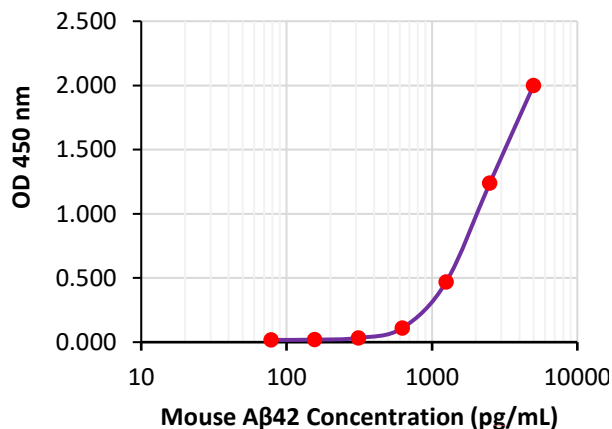
This assay recognizes natural and recombinant mouse A $\beta$ 42.

No cross-reactivity with others.

### RELATIVE PRODUCTS

Human p-Tau-217 ELISA (TBS3293)  
 Human p-Tau-181 ELISA (TBS3294)  
 Human Total Tau ELISA (TBS3295)  
 Human p-Tau-231 ELISA (TBS3296)  
 Human AD7 Human AD7C NTP (TBS3297)  
 Human Amyloid  $\beta$ 40 ELISA (TBS3298)  
 Human NF-L ELISA (TBS32101)  
 Human Total Amyloid  $\beta$  ELISA (TBS32104)  
 Human Gamma H2AX ELISA (TBS3265)  
 Human H2AX ELISA (TBS3266)  
 Human IL-4 ELISA (TBS3221)  
 Human IL-4 ELISA (TBS3221)  
 Human IL-6 ELISA (TBS3223)  
 Human IL-7 ELISA (TBS3224)  
 Human IL-8 ELISA (TBS3225)  
 Human IL-10 ELISA (TBS3226)  
 Human IL-13 ELISA (TBS3227)  
 Human IL-17 ELISA (TBS3228)  
 Human IL-22 ELISA (TBS3229)  
 Human IL-33 ELISA (TBS4245)  
 Human IFN-gamma ELISA (TBS3230)  
 Human TGF-  $\beta$ 1 ELISA (TBS3232)  
 Human GM-CSF ELISA (TBS3233)  
 Human MIP-1 $\alpha$  ELISA (TBS3234)  
 Protein Cell Lysis Buffer (catalog# TBS5001)  
 Protein Assay Kit (Catalog# TBS2005)  
 TMB Substrate System (Catalog#TBS5021)

**Fig.3 Mouse Amyloid  $\beta$  42 / A $\beta$ 42 Standard Curve**



**For research use only. Not for use in diagnostic procedures.**