

For the quantitative determination of mouse pTau-217 concentrations in cell culture supernates, serum, and plasma.

INTRODUCTION

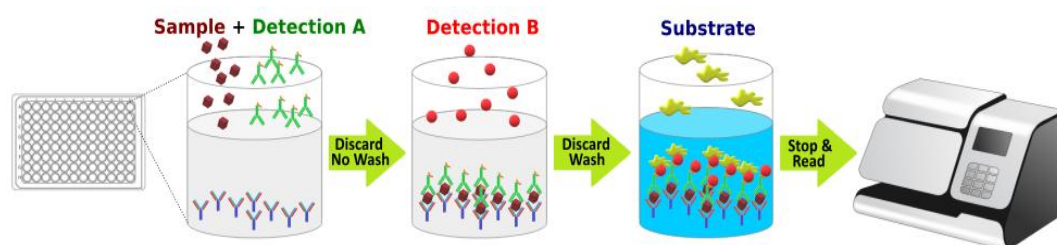
Phospho-tau217 (p-Tau217) is one of the most robust plasma biomarkers for Alzheimer's disease. In many studies, the marker identified people with the highest likelihood of Alzheimer's disease, almost 100 percent accuracy. In differential diagnosis, plasma p-Tau217 also distinguished AD from other neurodegenerative diseases, notably including tauopathies, with high accuracy, beating out other plasma markers including neurofilament light and p-Tau181.

Tribio Mouse Phospho Tau Thr217 (p-Tau217) ELISA is designed to quantitatively detect mouse p-Tau217 levels in serum, plasma, cerebrospinal fluid, and tissue homogenates. The main feature of this kit is that it applies a novel one-step detection strategy that integrates sample incubation and signal development, simplifying the traditional multi-step ELISA procedure. This makes the assay simple, fast, accurate, and easy to operate, with hands-on time within approximately 2 hours instead of 4–5 hours (Fig. 1). The assay provides a broad and reliable detection range suitable for physiological and pathological samples. The levels of mouse p-Tau217 in samples show good linearity and parallelism with the standard curve generated by the kit standards. Therefore, this kit can be used for quantitative determination of endogenous mouse p-Tau217 protein levels and related biomarker studies.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique (Fig. 1). A monoclonal antibody specific to mouse p-Tau217 was pre-coated onto a microplate. Standards or samples and Detection Antibody are pipetted into the wells, and concurrently incubated for 2 hours. Then, just aspirate each well, no wash, directly add Secondary Antibody, incubate the complex. Following a wash to remove any unbound antibody and samples, an **ultra-sensitive TMB substrate solution** is added to the wells for color develops. The color intensity is in proportion to the amount of bound in the initial step. The intensity of the color is measured by plate reader at 450 nm.

Fig.1



KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Mouse pTau-217 Microplate	TBS3005A	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse p-Tau217.	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 pTau-217 Standard	TBS3005B	50 µL of Recombinant mouse p-Tau217 (2.43 µg/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	TBS3005C	2.1 mL of mouse p-Tau217 Detection antibody.	May be stored for up to 4 months at 2-8 °C.
Detection B	TBS3005D	20 µL of HRP conjugated secondary antibody.	
Assay Diluent	TBS3005E	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, eye, and face protection. Wash hands thoroughly after handling.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

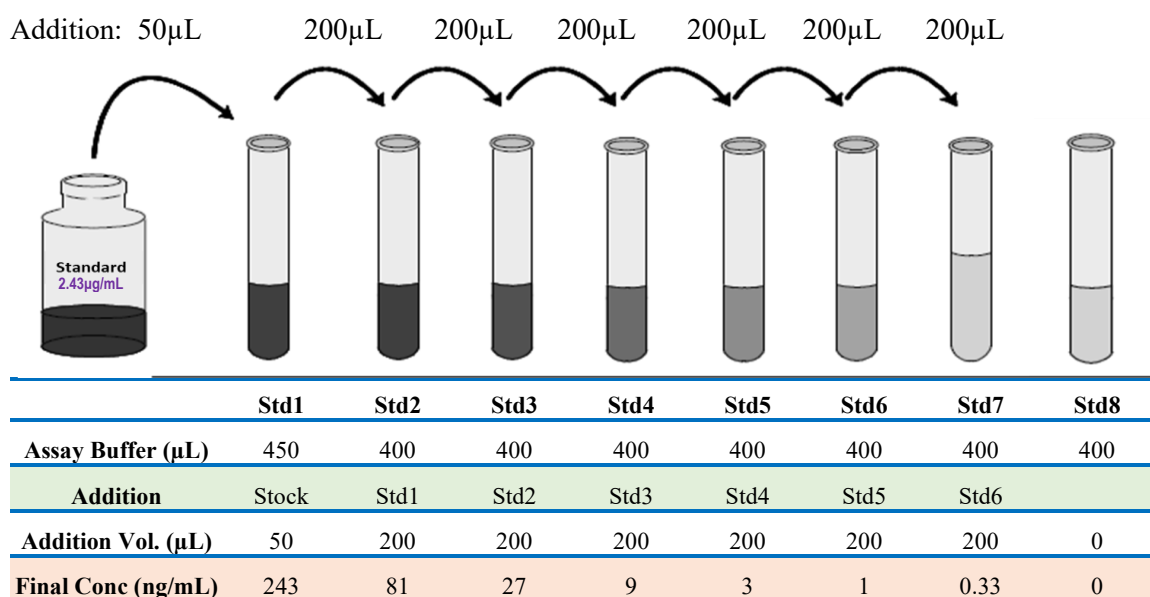
Wash Buffer: Add 12 mL of Wash Buffer Concentrate (10x) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved*).

Detection B working solution preparation: Add 12 μ L of Detection B HRP conjugated secondary antibody to 12 mL Assay Diluent to prepare Detection B working solution.

Mouse p-Tau217 Standard Preparation: Label test tubes as #1 through #8. Pipet 450 μ L of 1x Assay Diluent into tube #1, and 400 μ L into tubes #2 to #8 as diagram below.

1. Add 50 μ L of the Mouse p-Tau217 Standard stock solution (2.43 μ g/mL) to tube #1 and mix.
2. Make 3x serial dilutions of the standard using the Tube#1(243 ng/mL standard solution) from Tube #2 through #7 with sequential transfer of 200 μ L to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 243, 81, 27, 9, 3, 1 and 0.33 ng/mL. Tube# 8 is Standard 8 (0 ng/mL)

Fig.2 Diagram for Mouse pTau-217 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 μ L of standard, sample, or control per well.
2. Add 20 μ 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 μ 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 μ 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 μ L of **TMB Substrate** to each well. Incubate **at RT for 10 – 20 minutes** (*Protect from light*). The color becomes blue.
7. Add 50 μ 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 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will be 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 mouse is typically 200 pg/ml.

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

SPECIFICITY

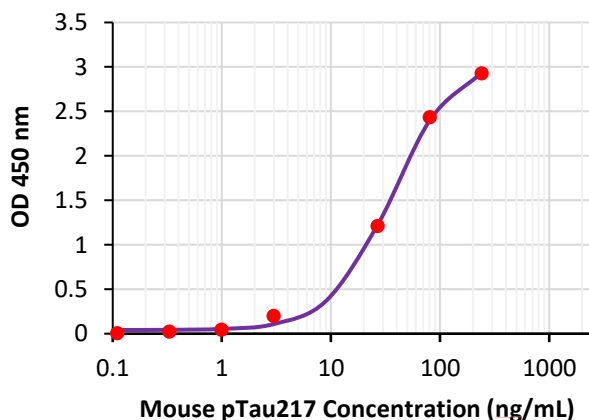
This assay recognizes natural and recombinant mouse pTau-217.

No cross-reactivity with others.

RELATIVE PRODUCTS

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 β 40 ELISA (TBS3298)
 Human Amyloid β 42 ELISA (TBS3299)
 Human Total Amyloid Amyloid β ELISA (TBS32104)
 Human NF-L ELISA (TBS32101)
 Human IL-2 ELISA (TBS3220)
 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- γ ELISA (TBS3230)
 Human TGF- β 1 ELISA (TBS3232)
 Human GM-CSF ELISA (TBS3233)
 Human MIP-1 α ELISA (TBS3234)
 Protein Cell Lysis Buffer (catalog# TBS5001)
 Protein Assay Kit (Catalog# TBS2005)
 TMB Substrate System (Catalog#TBS5021)

Fig.3 Mouse pTau217 Standard Curve



For research use only.