Tribioscience

Fast Mouse Phospho-Tau Thr181 ELISA (TBS3006)

For the quantitative determination of mouse pTau-181 concentrations in CSF, brain tissue, serum, and plasma.

INTRODUCTION

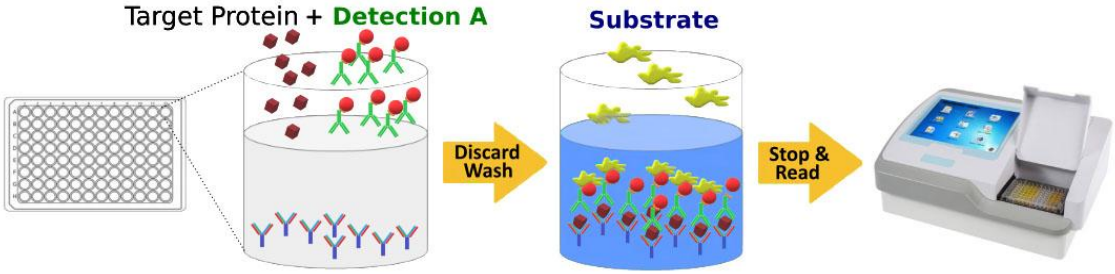
Phospho-tau181 (p-Tau181) is one of the most robust plasma biomarkers for Alzheimer's disease. Blood p-Tau181 can predict tau and amyloid β pathologies, differentiate Alzheimer's disease from other neurodegenerative disorders, and identify Alzheimer's disease across the clinical continuum. Blood p-Tau181 could be used as a simple, accessible, and scalable test for screening and diagnosis of Alzheimer's disease.

Tribio Fast Mouse Phospho Tau Thr181 (p-Tau181) ELISA is designed for quantitative detection of mouse p-Tau181 in serum, plasma, and other biological samples. It uses a proprietary one-step detection method that simplifies the traditional protocol, making the assay fast, easy, and accurate. The total hands-on time is within 2 hours instead of 4–5 hours (Fig. 1). The detection range is 0.3–243 ng/mL, and mouse p-Tau181 levels show good linearity with the standard curve, enabling reliable quantification.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique (Fig. 1). A monoclonal antibody specific to mouse p-Tau181 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, following a wash to remove any unbound antibody and samples, an **ultra-sensitive TMB substrate solution** is added to the wells for color development. The color intensity is in proportion to the amount of bound p-Tau181in the initial step. The intensity of the color is measured by plate read at 450 nm.

Fig. 1 Assay Principle



KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Mouse pTau-181 Microplate	TBS3006A	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for mouse p-Tau181.	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-181 Standard	TBS3006B	80 µL of Recombinant mouse p-Tau181 standard (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	TBS3006C	25 µL of p-Tau-181 mAb-HRP Conjugate (100x).	
Assay Diluent	TBS3006D	15 mL of a buffered protein base with preservatives.	May be store for up to 3 months at 2-8 °C
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.

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PRECAUTIONS

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

REAGENT PREPARATION

Bring all reagents to room temperature before use.

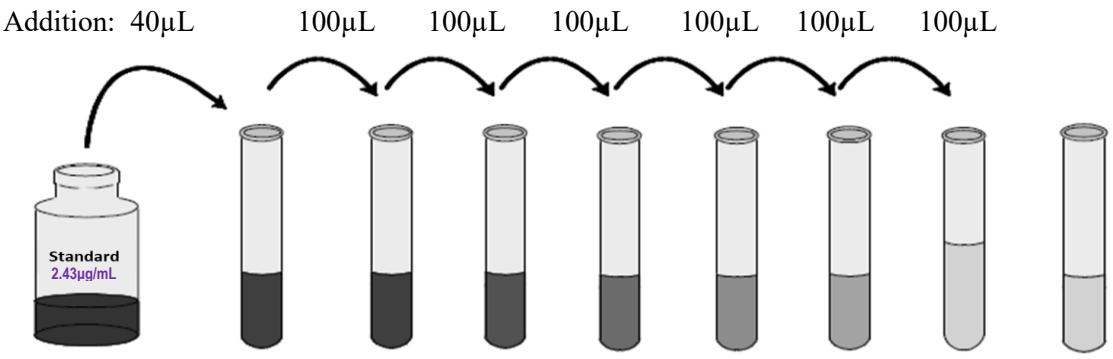
Wash Buffer: Add 12 mL of Wash Buffer Concentrate (10x) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved).*

Detection A working solution preparation: Add 21 µL of **Detection A** to 2.1 mL Assay Diluent with 100-fold dilution ratio to prepare Detrection A working solution.

Mouse p-Tau181 Standard Preparation: Label test tubes as #1 through #8. Pipet 360 µL of 1x Assay Diluent into tube #1, and 200 µL into tubes #2 to #8 **as diagram below.**

1. Add 40 µL of the Mouse p-Tau181 Standard stock solution (2.43 µg/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 100 µL 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-181 standard preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	360	200	200	200	200	200	200	200
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	40	100	100	100	100	100	100	0
Final Conc (ng/mL)	243	81	27	9	3	1	0.33	0

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 µL of standard, sample, or control per well.
2. Add 20 µL of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT with shaking for 2 hours**.
3. Aspirate each well, and wash for 3 times by filling each well with 200 µL 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.
4. Add 100 µL of **TMB Substrate** to each well. Incubate **at RT for 10 - 20min** (*Protect from light*). The color becomes blue**.**
5. Add 50 µL 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).
6. 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 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 ptau-181 is typically 300 pg/ml.
The Intra-assay CV and the Inter-assay CV are <10%.

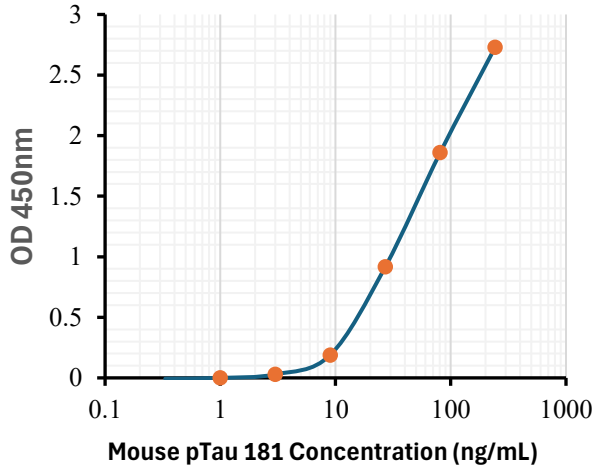
SPECIFICITY

This assay recognizes natural and recombinant mouse pTau-181.
No cross-reactivity with others.

RELATIVE PRODUCTS

Human p-Tau-217 ELISA (TBS3293)
Human Total Tau ELISA (TBS3295)
Human p-Tau-231 ELISA (TBS3296)
Human AD7 Human AD7C NTP (TBS3297)
Human Amyloid ß40 ELISA (TBS3298)
Human Amyloid ß42 ELISA (TBS3299)
Human NF-L ELISA (TBS32101)
Human Total Amyloid Amyloid ß ELISA (TBS32104)
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- ß1 ELISA (TBS3232)
Human GM-CSF ELISA (TBS3233)
Human MIP-1α ELISA (TBS3234)
Protein Cell Lysis Buffer (TBS5001)
Protein Assay Kit (TBS2005)
TMB Substrate System (TBS5021)
HRP Fluoresce Substrate System (TBS5026)

Fig 3 Mouse pTau 181 Standard Curve



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