

Fast Human Phospho-Tau Thr181 ELISA

For the quantitative determination of human pTau-181 concentrations in cell culture supernates, serum, and plasma.

INTRODUCTION

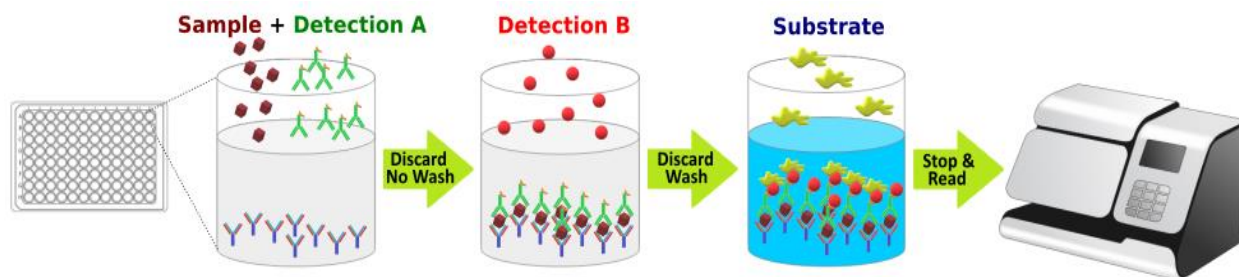
Phospho-tau181 (p-Tau181) is one of the most robust plasma biomarkers for Alzheimer’s disease. Blood p-Tau181 can predict tau and amyloid β pathologies, differentiate Alzheimer's disease from other neurodegenerative disorders, and identify Alzheimer's disease across the clinical continuum. Blood p-Tau181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

Tribioscience’s Fast Human Phospho Tau Thr181 (p-Tau181) ELISA is designed to quantitatively detect human p-Tau181 levels in serum, plasma, and other biological samples. The main feature is that **the kit uses our novel proprietary approaches to combine samples and detections into a one-step instead of the complicated traditional methods. It makes the assay simple, easy, accurate and fast. The hands-on time can be within 2 hours, not need 4-5 hours (Fig. 1). The detection range is from 0.3 to 243 ng/mL.** The levels of human p-Tau181 samples are parallel to the standard curves obtained using the kit standards linearly. Therefore, the kit can be used to determine relative mass values for natural human p-Tau181 protein.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique (See Fig. 1). A polyclonal antibody specific for human p-Tau181 was pre-coated onto a microplate. Standards or samples and Detection Antibody are pipetted into the wells, and concurrently incubated for 3 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 read at 450 nm.

Fig. 1 Assay Principle



KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Human pTau-181 Microplate	TBS3294A	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for human 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.
Human pTau-181 Standard	TBS3294B	50 μ L of Recombinant human p-Tau181 standard (2.43 μ g/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	TBS3294C	2.1 mL of human p-Tau181 biotinylated Detection antibody.	May be stored for up to 3 months at 2-8 °C.
Detection B	TBS3294D	400 μ L of streptavidin HRP.	
Assay Diluent	TBS3294E	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.

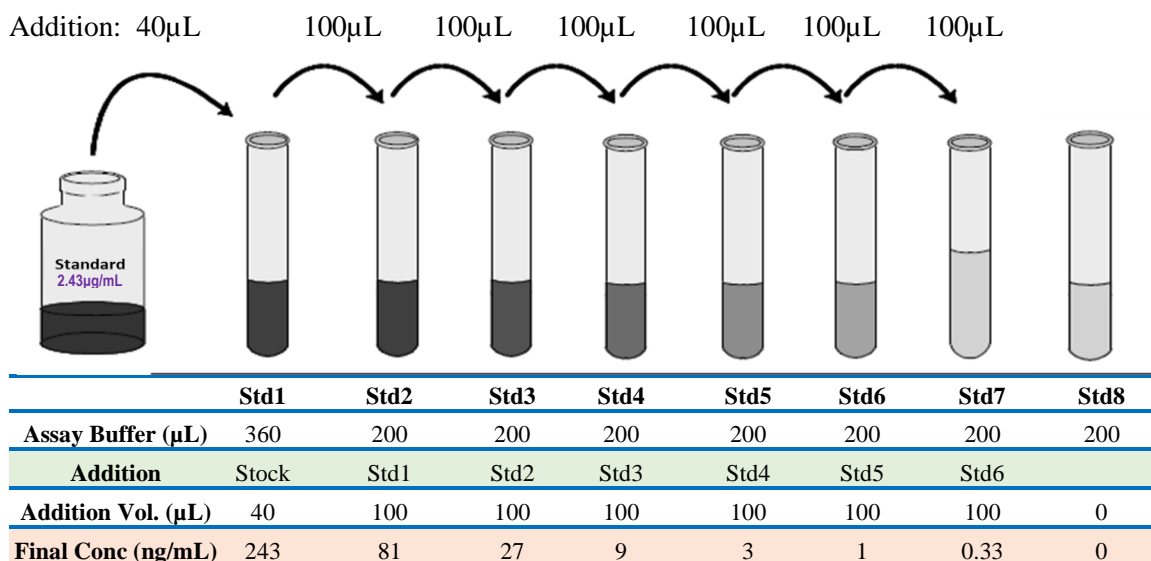
Wash Buffer: Add 12 mL of Wash Buffer Concentrate (10x) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved*).

Detection B working solution preparation: Add 240 µL of **Detection B** streptavidin-HRP to 12 mL Assay Diluent to prepare Detection B working solution.

Human 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 Human 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 Human pTau-181 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 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 3 hours**.
3. Aspirate each well (no wash). Invert the plate and blot it against clean paper towels.
4. Add 100 µL of Detection B working solution to each well. Incubate at **RT with shaking for 1 hour**.
5. Aspirate each well, and wash for 3 times by filling each well with 300 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.
6. Add 100 µL of **TMB Substrate** to each well. Incubate **at RT for 10 - 20min** (*Protect from light*). The color becomes blue.
7. 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).
8. Determine the optical density of each well within 20 minutes, using a microplate reader at 450nm. **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 human 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 (MOD) of human is typically 200 pg/ml.
The Intra-assay CV and the Inter-assay CV are <10%.

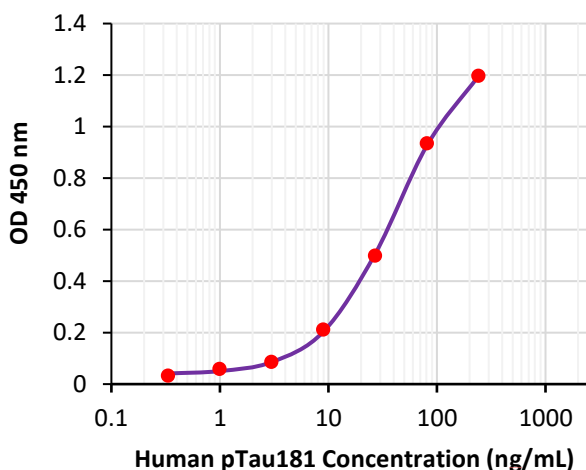
SPECIFICITY

This assay recognizes natural and recombinant human 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 β 40 ELISA (TBS3298)
- Human Amyloid β 42 ELISA (TBS3299)
- Human NF-L ELISA (TBS32101)
- Human Total Amyloid Amyloid β 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- β 1 ELISA (TBS3232)
- Human GM-CSF ELISA (TBS3233)
- Human MIP-1 α ELISA (TBS3234)
- Protein Cell Lysis Buffer (TBS5001)
- Protein Assay Kit (TBS2005)
- TMB Substrate System (TBS5021)
- HRP Fluoresce Substrate System (TBS5026)

Fig.3 Human pTau181 Standard Curve



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