

## Fast Human Phospho-Tau Thr231 ELISA

For the quantitative determination of human phosphorylated tau-231 protein concentrations in CSF, blood and cell culture supernates.

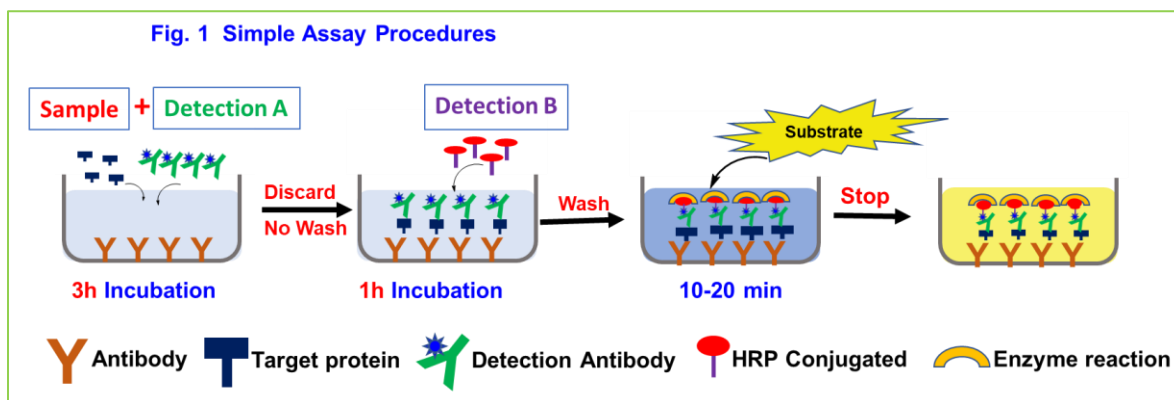
### INTRODUCTION

Tau is the major microtubule associated protein (MAP) of a mature neuron. An established function of MAPs is their interaction with tubulin and promotion of its assembly into microtubules and stabilization of the microtubule network. In Alzheimer disease (AD) brain tau is three to four-fold more hyperphosphorylated than the normal adult brain, resulting in the destabilization of microtubules in AD. Threonine 231 is one of the phosphorylation sites of human tau protein, and one of the early AD biomarkers.

Tribioscience's Fast Human Phospho Tau Thr231 (pT-231) ELISA is designed to quantitatively detect human pT-231 levels in CSF, 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 3 hours, not need 5-6 hours (Fig. 1). The detection range is from 0.3 to 243 ng/mL.** The levels of human pT-231 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 pT-231 protein.

### PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique (See Fig. 1). A monoclonal antibody specific for human pT-231 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 Streptavidin-HRP, 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.



### KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Human PTau-231 Microplate	TBS3296A	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human pT-231.	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-231 Standard	TBS3296B	60 µl of Recombinant human pT-231 (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	TBS3296C	2.1 ml of biotin human pT-231 Detection antibody.	May be stored for up to 3 months at 2-8 °C.
Detection B	TBS3296D	12 ml of Streptavidin-HRP	
Assay Diluent	TBS3296E	12 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	6ml 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**

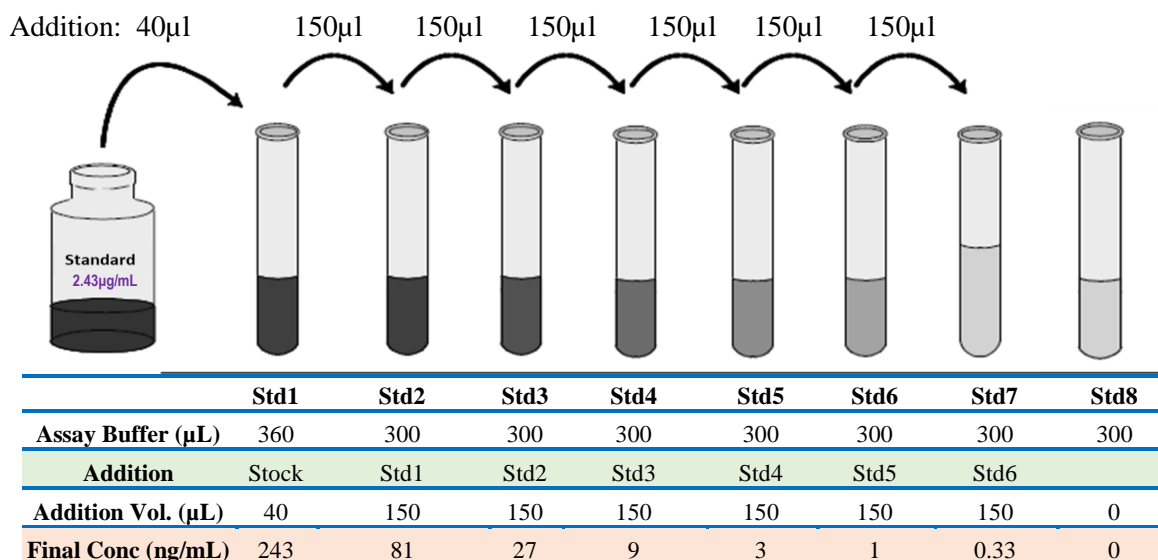
**Bring all reagents to room temperature before use.**

**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*).

**Human pT-231 Standard Preparation:** Label test tubes as #1 through #8. Pipet 360 µL of 1x Assay Diluent into tube #1, and 300 µL into tubes #2 to #8 **as diagram below**.

1. Add 40 µL of the Human pT-231 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 150 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 0.

**Fig.2 Diagram for Human pTau-231 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 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 to each well. Incubate at **RT 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 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 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%.

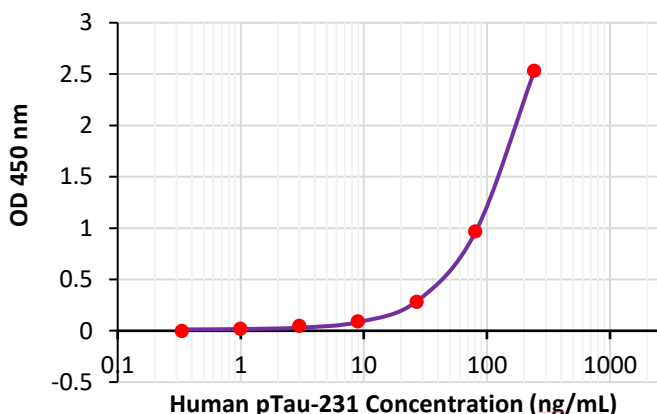
**SPECIFICITY**

This assay recognizes natural and recombinant human pTau-231. No cross-reactivity with others.

**RELATIVE PRODUCTS**

- Human IL-18 ELISA (TBS3219)
- 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 VASN ELISA (TBS4246)
- 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)
- Human p-Tau-181 ELISA (TBS3294)
- Human MAPT/Tau (total) ELISA (TBS3295)
- Human Thr217 (p-T217) ELISA (TBS3293)

Fig.3 Human pTau-231 Standard Curve



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