Fast Human MAPT/Tau (total) ELISA

For the quantitative determination of human Tau (Total) concentrations in CSF, Blood, and culture supernates.

INTRODUCTION

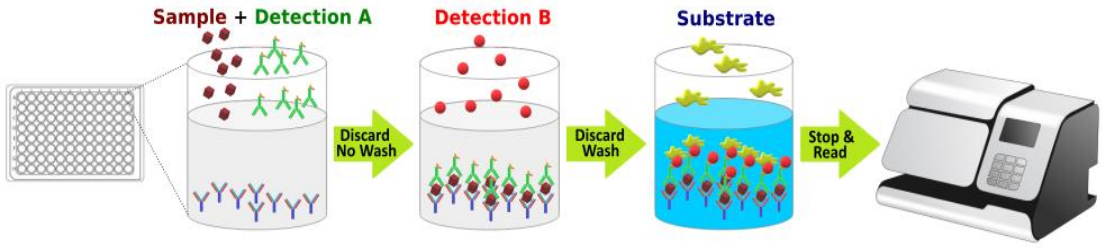
Tau is the major microtubule associated protein (MAP) of a mature neuron. An established function of MAPs is their interaction with tubulin and promotion of its assembly into microtubules and stabilization of the microtubule network. In Alzheimer disease (AD) brain tau protein concentration is increased more for several times than the normal adult brain. The quantitative detection of tau protein concentration can be assistant to patient selection for clinical studies and the development of new drugs and diagnostics for AD.

Tribioscience's Fast Human Tau (Total) ELISA is designed to quantitatively detect human Tau (Total) 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 2 hours, with no need for 4-5 hours (Fig. 1). The detection range is from 8000 to 125 pg/mL. The levels of human Tau (Total) protein in 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 Tau (Total) protein.

PRINCIPLE OF THE ASSAY

This assay employs our novel proprietary sandwich enzyme immunoassay techniques (see Fig. 1). A monoclonal antibody specific for human tau 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 tau bound in the initial step. The intensity of the color is measured by plate reading at 450 nm.

Fig. 1 Assay Principle



KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Human Tau (Total) Microplate	TBS3295A	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Tau (Total).	Return unused wells to the foil pouch. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.
Human Tau (Total) Standard	TBS3295B	30 µL of Recombinant human Tau (Total) (400 ng/mL).	Aliquot and store at -20 °C for up to 1 month in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS3295C	2.1 mL of human Tau (Total) Detection antibody.	May be stored for up to 3 months at 2-8 °C.
Detection B	TBS3295D	400 µL of Streptavidin-HRP	
Assay Diluent	TBS3295E	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 ℃. 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.

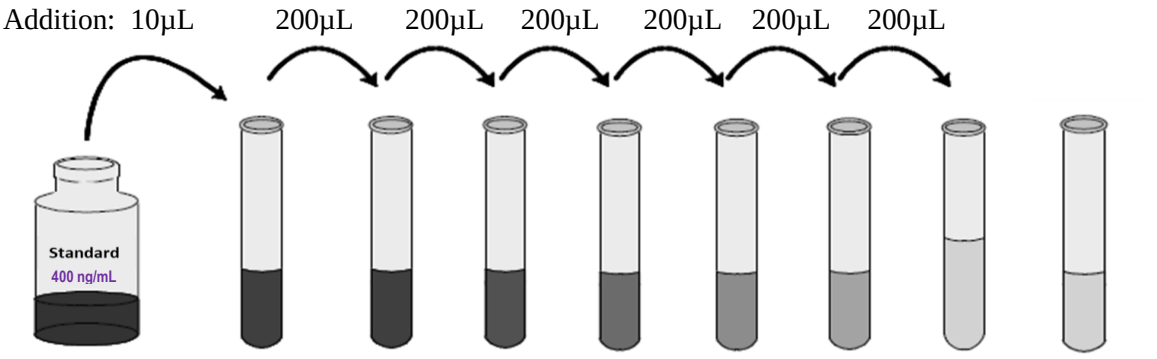
Wash Buffer: Add 12 mL of Wash Buffer Concentrate (10x) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved).*

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

Human Tau (Total) Standard Preparation: Label test tubes as #1 through #8. Pipet 490 µL of 1x Assay Diluent into tube #1, and 200 µL into tubes #2 to #8 **as diagram below.**

1. Add 10 µL of the Human Tau (Total) Standard stock solution (400 ng/mL) to tube #1 and mix.
2. Make 2 x serial dilutions of the standard using the Tube#1(8000 pg/mL standard solution) from Tube #2 through #7 with sequential transfer of 200 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 8000, 4000, 2000, 1000, 500, 250, and 125 pg/mL. Tube# 8 is Standard 8 (0 pg/mL).

Fig.2 Diagram for Human Tau (Total) standard preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	490	200	200	200	200	200	200	200
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	10	200	200	200	200	200	200	0
Final Conc (pg/mL)	8000	4000	2000	1000	500	250	125	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 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 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 Tau total is typically 125 pg/ml.
The Intra-assay CV and the Inter-assay CV are <10%.

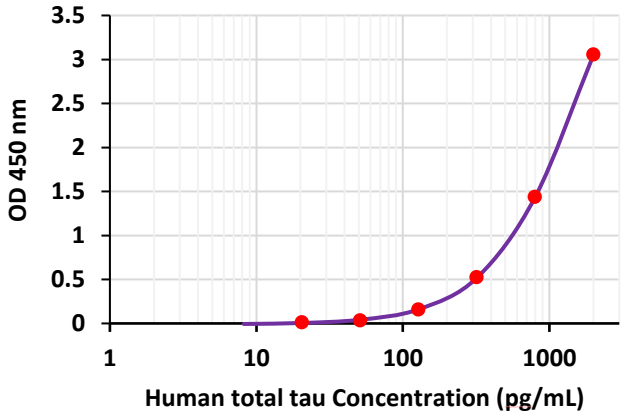
SPECIFICITY

This assay recognizes natural and recombinant human tau total.
No cross-reactivity with others.

RELATIVE PRODUCTS

Human IL-1ß 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- ß1 ELISA (TBS3232)
Human GM-CSF ELISA (TBS3233)
Human MIP-1α 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 Thr231 (p-T231) ELISA (TBS3296)
Human Thr217 (p-T217) ELISA (TBS3293)

Fig.3 Human total tau Standard Curve



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