INTRODUCTION

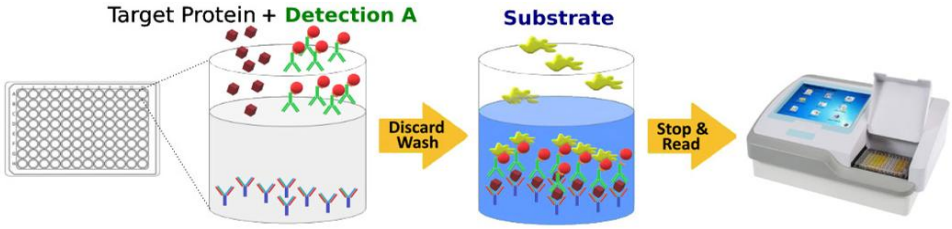
TAR DNA-binding protein 43 (TDP-43) is a 43-kDa protein encoded by the TARDBP gene. Under physiological conditions, TDP-43 is primarily localized in the nucleus, where it plays essential roles in RNA metabolism, microRNA biogenesis, and through the regulation of RNA targets, contributes to protein quality control and mitochondrial homeostasis. Under pathological conditions, however, TDP-43 can mislocalize to the cytoplasm, where it forms hyperphosphorylated and ubiquitinated aggregates. TDP-43 pathology has been associated with several neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS), frontotemporal lobar degeneration (FTLD), Huntington's disease, Niemann-Pick disease, and Alzheimer's disease.

The Tribioscience Fast Human TDP-43 ELISA is designed to quantitatively detect Human TDP-43 levels in different tissues including neural tissue, CSF, serum, 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 measurement can be finished in 3 hours, not needing 4-5 hours (Fig. 1). The detection range is 4 to 1000 ng/mL. The levels of human TDP-43 samples are parallel to the standard curves obtained using the kit standards linearly. These results indicate that this kit can be used to determine relative mass values for natural human TDP-43 protein.

PRINCIPLE OF THE ASSAY

This assay employs our novel proprietary sandwich enzyme immunoassay techniques (see Fig. 1). A monoclonal antibody specific to human TDP-43 is pre-coated onto a microplate. Standards or samples and HRP-conjugated detection antibody are pipetted into the wells and concurrently incubated to form a sandwich complex in one step. 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 TDP-43 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 TDP-43 Microplate	TBS32118A	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific to human TDP-43.	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 TDP-43 Standard	TBS32118B	7 µL of Recombinant human TDP-43 protein (100 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	TBS32118C	10 µL of Human TDP-43 antibody HRP (300x).	May be stored for up to 3 months at 2-8 °C.*
Assay Diluent	TBS32118E	20 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.).*

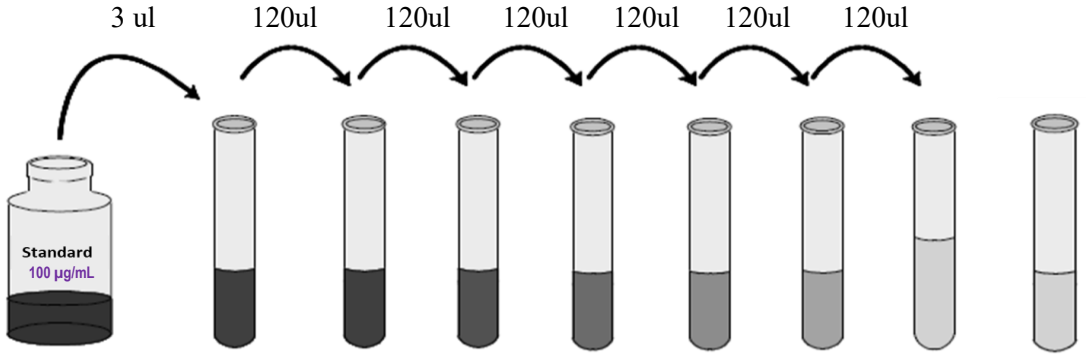
Detection A working solution preparation: Add 7 µL of Detection A Stock (HRP-human TDP-43 antibody) to 2093 µL Assay Diluent to prepare Detrection A working solution. Add 20 µL to each well.

Human TDP-43 Standard Preparation:

Label test tubes as #1 through #8. Pipet 297 µL of 1x Assay Diluent into tube #1, and 180 µL into tubes #2 to #7 as diagram below (Fig.2).

1. Add 3 µL of the Human TDP-43 Standard stock solution (100 µg/mL) by dilution of 100X to tube #1 (1000 ng/mL), and mix.
2. Make 2.5x serial dilutions of the standard using the 1000 ng/mL standard solution (tube #1) from tube #2 through #7 with sequential transfer of 120 µL to the next concentration. Mix each tube thoroughly and gently before the next transfer. The standard concentration in tubes 1 through 7 will be 1000, 400, 160, 64, 25.6, 10.2, and 4.1 ng/mL. Tube# 8 is Standard 0 as blank.

Fig.2: Diagram for Human TDP-43 standard preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	297	180	180	180	180	180	180	180
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	3	120	120	120	120	120	120	0
Final Conc (ng/mL)	1000	400	160	64	25.6	10.2	4.1	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 gently. Cover with the adhesive sealer. Incubate at 37°C for 2 hours (*Protect from light*).
3. 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.
4. Add 100 µL of TMB Substrate to each well. Incubate at RT for 10-20 min (*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 be corrected 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 TDP-43 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 (R2=0.9995) 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 human TDP-43 is typically 4 ng/mL.
The Intra-assay CV is 3.79% and the Inter-assay CV is <10%.

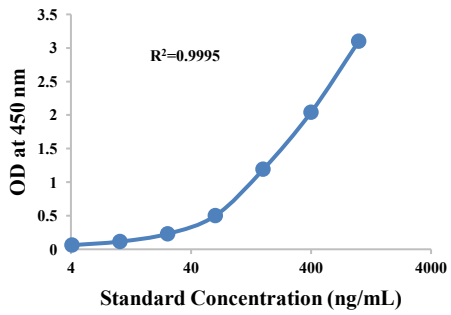
SPECIFICITY

This assay recognizes natural and recombinant human TDP-43.

RELATIVE PRODUCTS

Human IL-1β 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 IFN-gamma ELISA (TBS3230)
Human TGF- ß1 ELISA (TBS3232)
Human GM-CSF ELISA (TBS3233)
Human MIP-1α ELISA (TBS3234)
Human PD-1 ELISA (TBS32116)
Human PD-L1 ELISA (TBS32117)

Fig.3: Human TDP-43 Standard Curve



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