Fast Mouse Tyrosine Hydroxylase / TH ELISA

For the quantitation of mouse tyrosine hydroxylase concentrations in cell culture supernates, serum, plasma, and tissues.

INTRODUCTION

Tyrosine hydroxylase (TH) is the rate-limiting enzyme in the synthesis of the catecholamines dopamine, epinephrine and norepinephrine. Therefore, the regulation of the TH enzyme represents the central means for controlling the synthesis of these important catecholamines. TH is associated with the pathogenesis of several neurological and psychiatric diseases, including Parkinson's disease, dystonia, schizophrenia, affective disorders, and cardiovascular diseases. Therefore, a fast, novel quantitative method to monitor the changes in TH expression in disease models facilitates the identification and characterization of neuro-modulatory and neuroprotective therapeutic agents.

The Tribio® Fast Mouse Tyrosine Hydroxylase/TH ELISA is a solid-phase ELISA designed to measure mouse TH levels in cell culture supernatants, serum, plasma, and tissues. The kit uses our novel proprietary ELISA technology to combine sample and detection into a one-step, making the assay simple, easy, accurate and fast. The entire procedure can be finished in 2 hours, not 5-6 hours (Fig. 1). The detection range is from 1 to 1000ng/mL. The levels of mouse TH samples are extrapolated from the standard curve using the standards provided with the kit.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. Monoclonal antibodies specific for mouse TH are pre-coated on the wells of a microplate. Standards and samples are pipetted into the wells and incubated with HRP-conjugated detection antibody specific for mouse TH. Following a wash to remove any unbound antibodies and samples, an ultra-sensitive TMB substrate solution is added to the wells for reaction to occur. The color intensity developed is in proportion to the amount of TH bound in the initial step. Results are obtained by measuring the color intensity of each well with a plate reader at 450 nm.

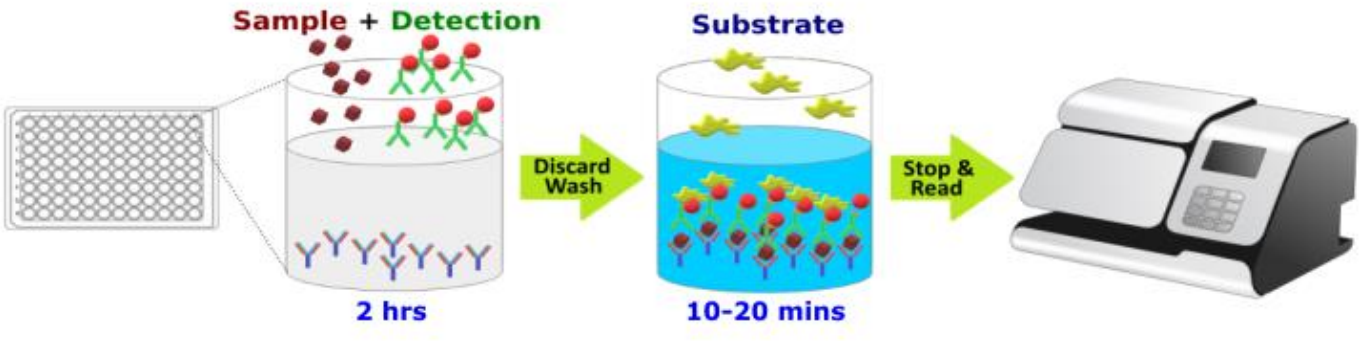


Fig. 1. Graphical representation of the novel proprietary ELISA approach.

KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE CONDITIONS
Mouse TH Coated Microplate	TBS3701A	96 well strip microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse TH.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.
Mouse TH Standard	TBS3701B	50 µL of recombinant mouse TH protein (7290ng/mL).	Aliquot and store at -20 °C for up to 1 month in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS3701C	2.1 ml of HRP-Mouse TH antibody.	May be stored for up to 3 months at 2-8 °C.*
Assay Diluent	TBS3701D	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	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 10 mL of Wash Buffer Concentrate (10x) to 90 mL of deionized distilled water to prepare 100 mL of Wash Buffer.

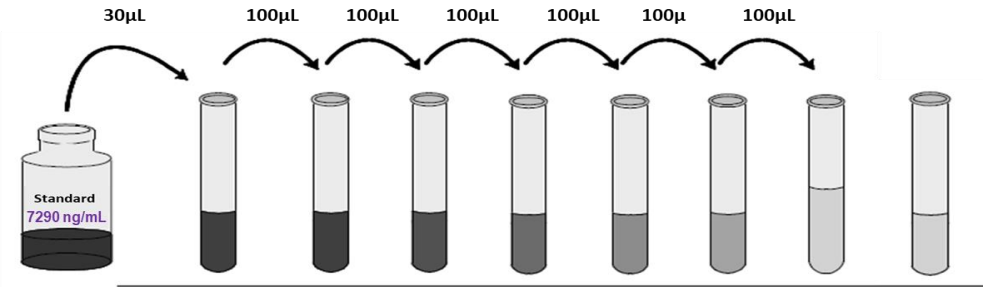
Mouse TH Standard Preparation:

1. Label test tubes as #1 through #8. Pipet 270 µL of 1x Assay Diluent into tube #1, and 200 µL into tubes #2 to #8 **as diagram below (Fig2.).**

2. Add 30 µL of the mouse TH Standard stock solution (7290ng/mL) by dilution of 10 times to tube #1 and mix completely.

3. Take 100 µL of the mouse TH standard from tube #1 to tube #2 and mix completely. Repeat 3 x serial dilutions for tubes #3 through #7. The standard concentration in tube 1 through 7 will be 729, 243, 81, 27, 9, 3 and 1 ng/mL. Tube# 8 is Standard 0.

Fig.2 Diagram for Mouse TH Standard Preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Diluent (µL)	**270**	**200**	**200**	**200**	**200**	**200**	**200**	**200**
Addition	**Stock**	**Std1**	**Std2**	**Std3**	**Std4**	**Std5**	**Std6**	
Addition Vol. ((µL)	**30**	**100**	**100**	**100**	**100**	**100**	**100**	
Std Conc (ng/mL)	729	243	81	27	9	3	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. Cover with the adhesive sealer. Incubate at **RT for 2 hours.**
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 10-20min** (*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 5 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.

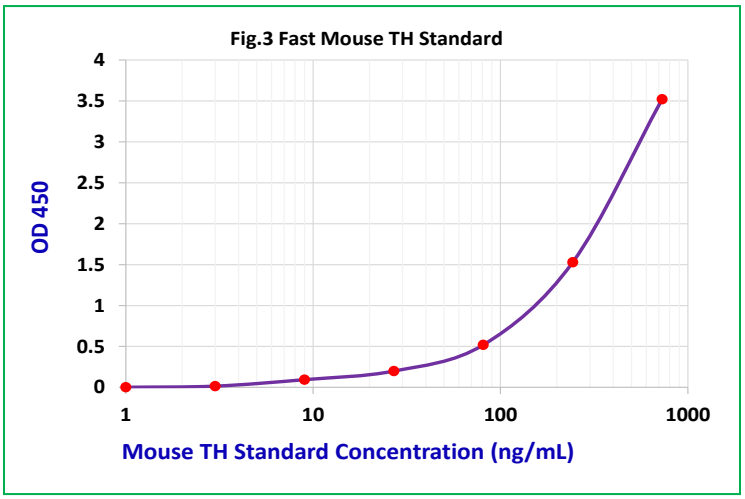
CALCULATION OF RESULTS

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 TH 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 is provided for demonstration only as Fig.3. A standard curve should be generated for each set of samples assayed.



SENSITIVITY

The minimum detectable dose (MOD) of mouse TH is typically 0.1 ng/ml.

SPECIFICITY

This assay recognizes natural and recombinant Mouse TH.

RELATED 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 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)

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