Fast Human TNF-alpha ELISA

For the quantitative determination of human tumor necrosis factor concentrations in cell culture supernates, serum, and plasma.

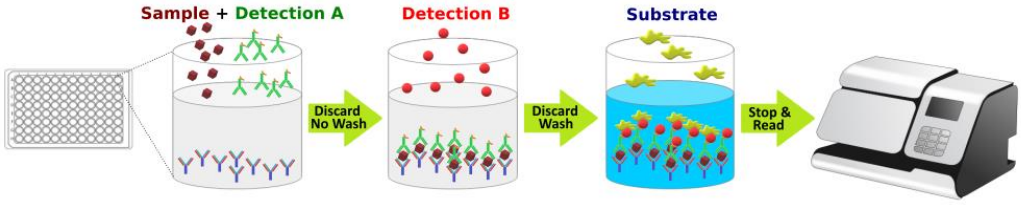
INTRODUCTION

Tumor necrosis factor alpha (TNF-α, also known as TNFSF2) is a potent mediator of immune and inflammatory response. It is produced by many activated cell types including monocytes, macrophages, astrocytes, granulocytes, T and B lymphocytes, NK cells, keratinocytes, fibroblasts, and certain tumor cells. TNF-α is involved in numbers of pathological conditions including inflammation, apoptosis, lipid metabolism, trauma, asthma, rheumatoid arthritis, pain, obesity septic shock, autoimmunity, and cancer.

The Tribo™ Fast Human TNF-α ELISA is designed to quantitatively detect Human TNF-α levels in different tissues including skin, muscle, neural, 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 need 4-5 hours (Fig. 1). **The detection range is from 8 to 2000pg/mL**. The levels of human TNF-α 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 TNF-α protein.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative e sandwich enzyme immunoassay technique (See Fig. 1). A monoclonal antibody specific for human TNF-α was pre-coated onto a microplate. Standards and samples are pipetted into the wells, and then, incubated with HRP-conjugated detection antibody specific for human TNF-α. 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 TNF-α 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 TNF-α Microplate	TBS3235A	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for human TNF-α.	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 TNF-α Standard	TBS3235B	30 µl of Recombinant human TNF-α protein (100ng/mL).	Aliquot and store at -20 °C for up to 1 month in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS3235C	2.1 ml of Biotin-Human TNF-α antibody.	May be stored for up to 3 months at 2-8 °C.*
Detection B	TBS3235D	12 ml of Streptavidin-HRP.	
Assay Diluent	TBS3235E	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.	
Plate Sealers	N/A	Adhesive strips.	

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.

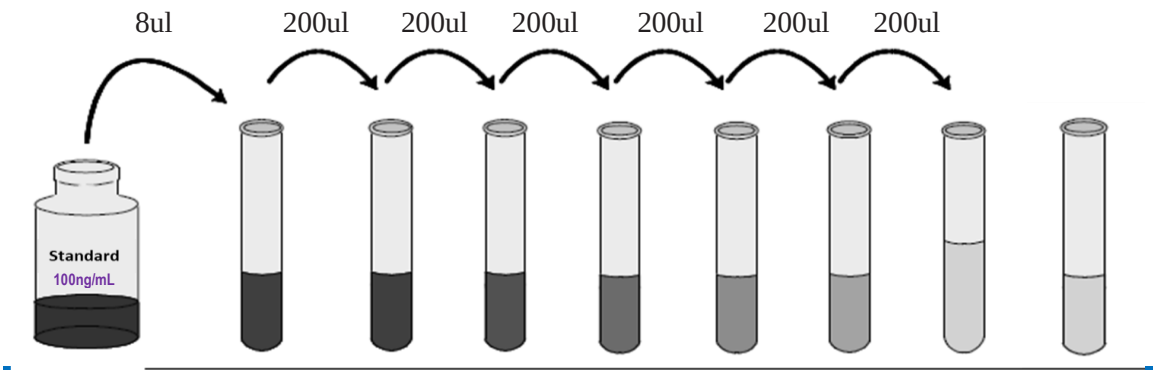
Wash Buffer: Add 12 mL of Wash Buffer Concentrate （10x） to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.).*

Human TNF-α Standard Preparation:

Label test tubes as #1 through #8. Pipet 392 µL of 1x Assay Diluent into tube #1, and 300 µL into tubes #2 to #8 **as diagram below.**

1. Add 8 µL of the Human TNF-α Standard stock solution (100ng/mL) by dilution of 50X to tube #1 and mix.
2. Make 2.5x serial dilutions of the standard using the 2000pg/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 2000, 800, 320, 128, 51.2, 20.48 and 8.192 pg/mL. Tube# 8 is Standard 0.

Fig.2 Diagram for Human TNF-α standard preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	**392**	300	300	300	300	300	300	300
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	8	200	200	200	200	200	200	0
Final Conc (pg/ml)	2000	800	320	128	51.2	20.48	8.192	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 hour**.
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 TNF-α 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=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 (MOD) of human TNF-α is typically 10pg/ml.
The Intra-assay CV is 3.79% the Inter-assay CV is <10%.

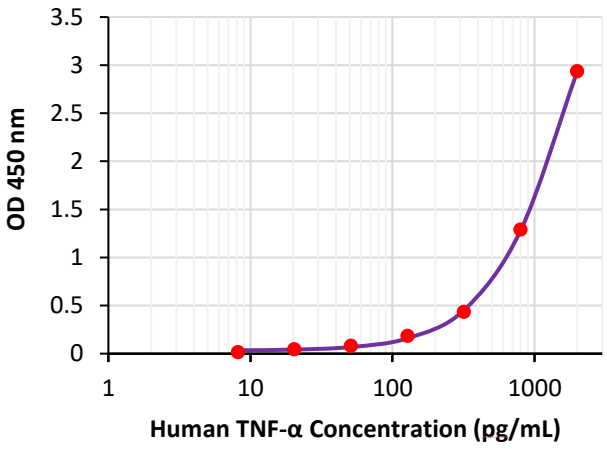
SPECIFICITY

This assay recognizes natural and recombinant human TNF-α.
No cross-reactivity: human TNF-β; human TNF RI, human TNF RII; Porcine TNF-α; Rat TNF-α; Mouse TNF-α; Mouse TNF RI, Mouse TNF RII.

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)

Fig.3 Human TNF-α Standard Curve



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