Tribioscience

Rat TNF-alpha ELISA Catalog Number: TBS3054

For the quantitative determination of rat TNF-α concentrations in cell culture supernatants, serum, and plasma.

INTRODUCTION

Tumor necrosis factor alpha is a pro-inflammatory cytokine produced primarily by activated macrophages, as well as by other immune cells such as T lymphocytes, natural killer (NK) cells, and mast cells. It plays a crucial role in the body's immune response by promoting inflammation, inducing fever, and regulating cell death (apoptosis). Excessive or unregulated TNF-α production is associated with chronic inflammatory and autoimmune diseases such as rheumatoid arthritis, psoriasis, and inflammatory bowel disease.

The Tribioscience Rat TNF-α ELISA is designed to quantitatively detect rat 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 62 to 4000 pg/mL**. The levels of rat 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 rat TNF-α protein.

Alternative names: TNF-α; Tumor Necrosis Factor α; TNF-alpha; TNF-a

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique (See Fig. 1). A monoclonal antibody specific for rat 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 rat 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.

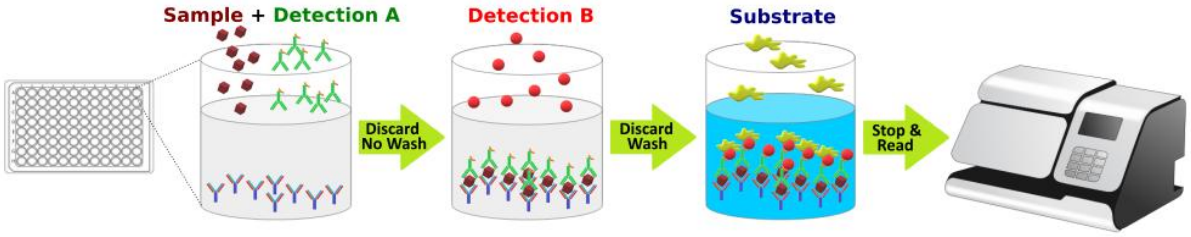


Fig.1: Simple ELISA procedure

KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Rat TNF-α Microplate	TBS3054A	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for rat TNF-α.	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.
Rat TNF-α Standard	TBS3054B	30 µl of Recombinant rat TNF-α protein (200 ng/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	TBS3054C	2.1 ml of Biotin-Rat TNF-α antibody.	May be stored for up to 3 months at 2-8 °C.
Detection B	TBS3054D	12 ml of Streptavidin-HRP.	
Assay Diluent	TBS3054E	12 ml of a buffered protein base with preservatives.	
WashBuffer	TBS3000W	12ml 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.

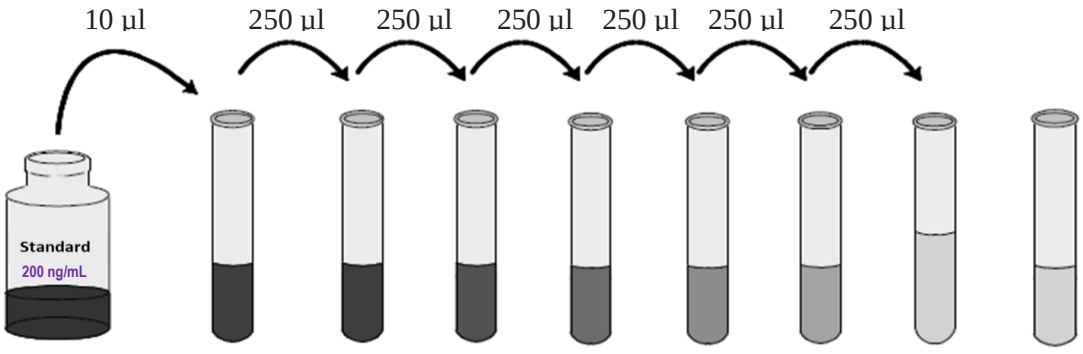
Wash Buffer: Add 12 mL of Wash Buffer Concentrate (10x) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.).*

Rat TNF-α Standard Preparation:

Label test tubes as #1 through #8. Pipet 490 µL of 1x Assay Diluent into tube #1, and 250 µL into tubes #2 to #8 **as diagram below.**

1. Add 10 µL of the Rat TNF-α Standard stock solution (200 ng/mL) by dilution of 50X to tube #1 and mix.
2. Make 2x serial dilutions of the standard using the 4000pg/mL standard solution from tube #2 through #7 with sequential transfer of 250 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 4000, 2000, 1000, 500, 250, 125 and 62.5 pg/mL. Tube# 8 is Standard 0.

Fig.2 Diagram for Rat TNF-α standard preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	490	250	250	250	250	250	250	300
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	10	250	250	250	250	250	250	0
Final Conc (pg/mL)	4000	2000	1000	500	250	125	62.5	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 (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 rat 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=0.9998) 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 Rat TNF-α is typically 10 pg/ml.
The Intra-assay CV is < 10% the Inter-assay CV is < 12%.

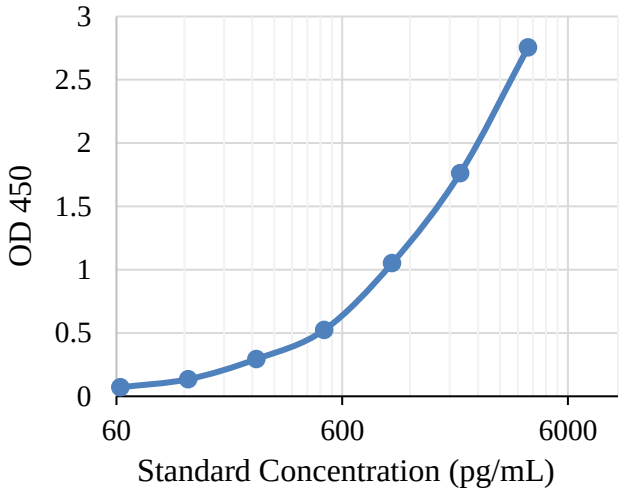
SPECIFICITY

This assay recognizes natural and recombinant rat TNF-α.
No cross-reactivity with others.

RELATIVE PRODUCTS

Mouse IL-1β ELISA (TBS3219)
Mouse IL-2 ELISA (TBS3220)
Mouse IL-4 ELISA (TBS3221)
Mouse IL-6 ELISA (TBS3223)
Mouse IL-7 ELISA (TBS3224)
Mouse IL-8 ELISA (TBS3225)
Mouse IL-10 ELISA (TBS3226)
Mouse IL-13 ELISA (TBS3227)
Mouse IL-17 ELISA (TBS3228)
Mouse IL-22 ELISA (TBS3229)
Mouse IFN-gamma ELISA (TBS3230)
Mouse TGF- ß1 ELISA (TBS3232)
Mouse GM-CSF ELISA (TBS3233)
Mouse MIP-1α ELISA (TBS3234)
Rat IL-1β ELISA (TBS3055)

Fig. 3: Rat TNF-α Standard Curve



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