



Instructions for Use

Human TNF- α ELISA

Q-TNF

Enzyme immunoassay for the quantitative determination of Human Tumor Necrosis Factor-alpha (TNF- α) in serum, plasma and cell culture supernatants

REF

TR-TNFv1



12X8



2-8°C

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

MATRIKS BIOTEK LABORATORIES

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1. INTENDED USE

The *Matriks Biotek* Human TNF-alpha Enzyme-Linked-Immuno-Sorbent-Assay (ELISA) Kit is intended for the quantitative determination of Tumor Necrosis Factor alpha (TNF- α) in human serum, plasma, and cell culture supernatants. TNF- α ELISA is for research use only.

2. SUMMARY AND EXPLANATION

Tumor necrosis factor- α (TNF- α) is a pleiotropic inflammatory cytokine. Most organs of the body appear to be affected by TNF- α , and the cytokine serves a variety of functions. The cytokine possesses both growth stimulating properties and growth inhibitory processes, and it appears to have self regulatory properties as well. TNF- α circulates throughout the body responding to stimuli (infectious agents or tissue injury); activating neutrophils; altering the properties of vascular endothelial cells; regulating metabolic activities of other tissues as well as exhibiting tumoricidal activity by inducing localized blood clotting. TNF- α also inhibits lipoprotein lipase activity resulting in cachexia, a physical wasting condition. Activation of B-cells by the Epstein Barr virus can be inhibited by TNF- α . Due to its varied actions throughout the immune system, TNF- α may play a role in the pathogenesis of many disease states.

Since TNF- α plays a role in several diseases, a substantial amount of research has been conducted concerning TNF- α therapies and anti-TNF- α therapies. As TNF- α exhibits anti-tumor activity, research has been conducted to determine the protein's effectiveness against certain forms of cancers. Utilizing TNF- α tumoricidal activities has proved problematic, especially due to the cytotoxin's systematic toxicity.

Measurement of TNF- α levels has also been shown to be useful in transplant research, TNF- α to be markedly elevated in renal allograft rejection episodes. Recent evidence has been presented on increased TNF- α levels in Bone Marrow Transplant (BMT). BMT patients with major transplant related complications such as interstitial pneumonitis and severe acute graft-versus-host disease had TNF- α levels significantly increase over controls.

3. TEST PRINCIPLE

The *Matriks Biotek* Human TNF- α ELISA is a sandwich assay for the determination of TNF- α in serum, plasma and cell culture supernatants. During the first incubation period, TNF- α in patient serum/plasma samples are captured by the monoclonal antibody to human TNF- α coated on the wall of the microtiter wells. After washing away the unbound components from samples, a peroxidase-labelled second monoclonal antibody conjugate is added to each well and then incubated. After a second washing step, the bound enzymatic activity is detected by addition of tetramethylbenzidine (TMB) chromogen-substrate. Finally, the reaction is terminated with an acidic stop solution. The intensity of the reaction color is directly proportional to the concentration of human TNF- α in sample.

4. WARNINGS AND PRECAUTIONS

1. For research use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood. For further information (clinical background, test performance, automation protocols, alternative applications, literature, etc.) please refer to the local distributor.
3. In case of severe damage of the kit package please contact Matriks Biotek or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guidelines or regulations.
8. Avoid contact with Stop solution. It may cause skin irritations and burns.
9. Some reagents contain sodium azide (NaN_3) as preservatives. In case of contact with eyes or skin, flush immediately with water. NaN_3 may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with large volume of water to avoid azide build-up.
10. All reagents of this test kit containing human serum or plasma have been tested and were found negative for HIV I/II, HBsAg and HCV by FDA approved procedures. However, a presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.

5. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters. The strips of microtiter plate is stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2-8°C.

6. SPECIMEN COLLECTION AND STORAGE

Serum, Plasma (EDTA, Heparin) Cell Culture Supernatant.

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Storage:	2-8°C	-20°C	Keep away from heat or direct sun light Avoid repeated freeze-thaw cycles
Stability:	2 d	>2 mon	

7. MATERIALS SUPPLIED

1 x 12 x 8	MTP	Microtiter Plate Break apart strips. Microtiter plate with 12 rows each of 8 wells precoated with monoclonal antibody to human TNF- α .
2 Vials (2x10 ng TNF/vial)	STANDARDS LYOPHILIZED	Standards lyophilized Each vial contains 10 ng of recombinant human TNF- α . Used for construction of the standard curve.
1 x 25 mL	ASSAY BUF	Assay Buffer Blue colored. Ready to use. Contains proteins and <0.1% NaN ₃ .
1 x 12 mL	POD CONJ	Peroxidase Conjugate Red colored. Ready to use. Contains peroxidase (POD) conjugate, stabilizer and preservatives.
1 x 12 mL	TMB SUBS	TMB Substrate Solution Ready to use. Contains TMB
1 x 12 mL	TMB STOP	TMB Stop Solution Ready to use. 1N HCl.
1 x 50 mL	WASHBUF CONC	Wash Buffer, Concentrate (20x) Contains Buffer with Tween-20.
2 x	ADH FILM	Adhesive Film For covering of Microtiter Plate during incubation.

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Bidistilled or deionised water
2. Micropipettes (< 3% CV) and tips to deliver 5-1000 μ L.
3. Calibrated measures.
4. Tubes (1 mL) for sample dilution in case of need.
5. Absorbent paper and timer.
6. Standard laboratory glass or plastic vials, cups, etc.
7. Wash bottle, automated or semi-automated microtiter plate washing system
8. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 620-690 nm is optional)

9. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.

3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each reagent, standard or specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. Use a pipetting scheme to verify an appropriate plate layout.
5. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
6. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
7. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

10. PRE-TEST SETUP INSTRUCTIONS

10.1. Preparation of Components

Dilute/ Dissolve	Component	With	Diluent	Relation	Remarks	Storage	Stability
10 mL	Wash Buffer*	Up to 200 mL	bidist. Water	1:20	Warm up at 37°C to dissolve crystals. Mix vigorously.	2-8 °C	4 w

*. Prepare Wash Buffer before starting assay procedure.

10.2. Preparation of Standards

Standards should be prepared before starting assay procedure. Reconstitute each lyophilized TNF- α standard vial with **1 ml of Assay Buffer** in order to prepare stock standard. Shake it gently avoiding massive foam formation and should be kept for 10 min at room temperature for complete dissolution.

Prepare stock standard just before use. Do not use reconstituted standard more than once.

After reconstitution, the concentration of **Stock Standard** is **10ng/ml**

Standard A (1000 pg/mL): 900 μ L of Assay Buffer + 100 μ L of Stock Standard.

Standard B (500 pg/mL): 250 μ L of Assay Buffer + 250 μ L of Standard A.

Standard C (250 pg/mL): 250 μ L of Assay Buffer + 250 μ L of Standard B.

Standard D (125 pg/mL): 250 μ L of Assay Buffer + 250 μ L of Standard C.

Standard E (62 pg/mL): 250 μ L of Assay Buffer + 250 μ L of Standard D.

Standard F (31 pg/mL): 250 μ L of Assay Buffer + 250 μ L of Standard E.

Standard G (Zero standard): Use only 250 μ L of Assay Buffer.

11. TEST PROCEDURE

1.	Pipette 50 µL of Assay Buffer non-exceptionally into each of the wells to be used.
2.	<p>Pipette 50 µL of each prepared Standards and serum/plasma or cell culture supernatant sample into the respective wells of microtiter plate.</p> <p><u>Wells</u></p> <p>A1: Standard A B1: Standard B C1: Standard C D1: Standard D E1: Standard E F1: Standard F G1: Standard G H1 and on: Sample (serum/plasma or cell culture supernatant)</p>
3.	Cover the plate with adhesive film. Briefly mix contents by gently shaking the plate. Incubate 120 min at room temperature (18-25°C).
4.	Remove adhesive film. Discard incubation solution. Wash plate 3 times each with 300 µL of diluted Wash Buffer . Remove excess solution by tapping the inverted plate on a paper towel.
5.	Pipette 100 µL of ready-to use Peroxidase Conjugate into each well.
6.	Cover the plate with adhesive foil. Incubate 60 min at room temperature (18-25°C).
7.	Remove adhesive film. Discard incubation solution. Wash plate 3 times each with 300 µL of diluted Wash Buffer . Remove excess solution by tapping the inverted plate on a paper towel.
8.	Pipette 100 µL of TMB Substrate Solution into each well.
9.	Incubate 20 min (without adhesive film) at room temperature (18-25°C) in the dark.
10.	Stop the substrate reaction by adding 100 µL of Stop Solution into each well. Color changes from blue to yellow. Briefly mix contents by gently shaking the plate.
11.	Measure optical density with a photometer at 450 nm within 30 min after pipetting of the Stop Solution.

12. QUALITY CONTROL

The test results are only valid only if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All standards must be found within the acceptable ranges as stated above. If the criteria are not met, the run is not valid and should be repeated. In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

13. CALCULATION OF RESULTS

13.1. Using the standards construct a standard curve by plotting the OD_{450 nm} for each of 6 standards (31, 62, 125, 250, 500, 1000 pg/mL) (zero standard could be omitted) on the vertical (Y-axis) axis versus the corresponding TNF- α concentration on the horizontal (X-axis) axis, thus creating a standard curve by 6 points obtained.

13.2. The concentration of the samples can be read directly from this standard curve. Using the absorbance value for each sample, determine the corresponding concentration of TNF- α from the standard curve. Find the absorbance value on the Y-axis and extend a horizontal line to the curve. At the point of intersection, extend a vertical line to the X-axis and read the TNF- α concentration for the unknown sample.

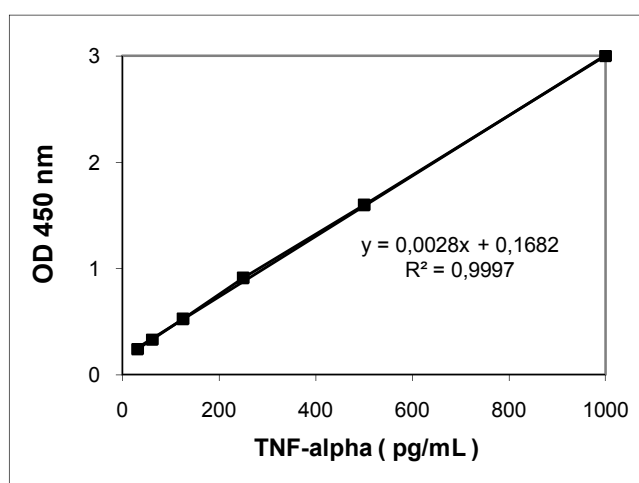
13.3. Any sample reading greater than the highest standard should be diluted appropriately with Assay Buffer and retested. Therefore, if the samples have been diluted, the concentration determined from the standard-curve must be multiplied by the dilution factor.

13.4. Automated method: Computer programs can also generally give a good fit.

Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	TNF- α (pg/mL)	Mean OD
A	1000	3.000
B	500	1.600
C	250	0.910
D	125	0.525
E	62	0.330
F	31	0.240
G	0	0.070



14. ASSAY CHARACTERISTICS

14.1. SPECIFICITY

This sandwich ELISA recognises both natural and recombinant human TNF- α . This kit exhibits no detectable cross-reactivity with human IL-1 β , IL-2, IL-4, IL-6, IL-10, IL-12, TGF- β , M-CSF, GM-CSF, EGF, IFN- α , IFN- γ , TNF- β and mouse TNF- α tested at concentrations up to 50 ng/mL.

14.2. SENSITIVITY

The lowest detectable level that can be distinguished from the zero standard is 7 pg/mL.

14.3. PRECISION OF KIT

Intra-assay CV: <10% for human TNF- α range of 31-1000 pg/mL.

Inter-assay CV: <10% for human TNF- α range of 31-1000 pg/mL.

14.4. RECOVERY

Recovery rate was found to be higher than 98% when spiked using normal human serum samples with known concentrations.

15. REFERENCES

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