

Human TNF α ELISA KIT

Catalog No. 850.090.096

INTENDED USE

The Human TNF α ELISA is to be used for the in-vitro quantitative determination of Tumor Necrosis Factor in human serum, plasma, buffered solutions or cell culture medium. The assay will recognize both natural and recombinant human TNF α . This kit recognizes both free and receptor (TNF-RI and TNF-RII) bound TNF α . **This kit has been configured for research use only and is not to be used in diagnostic procedures.**

PRINCIPLE OF THE METHOD

The TNF α Kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for TNF α has been coated onto the wells of the microtiter strips provided. Samples, including standards of known TNF α concentrations, control specimens and unknowns are pipetted into these wells.

During the first incubation, the TNF α antigen and a biotinylated monoclonal antibody specific for TNF α are simultaneously incubated.

After washing, the enzyme (streptavidin-peroxydase) is added. After incubation and washing to remove all the unbound enzyme, a substrate solution which is acting on the bound enzyme is added to induce a coloured reaction product. The intensity of this coloured product is directly proportional to the concentration of TNF α present in the samples.

REAGENTS PROVIDED AND RECONSTITUTION

REAGENTS (Store at 2-8°C)	QUANTITY	COLOUR CODE	RECONSTITUTION
96-wells microtiter plates	2		Ready-to-use
Plastic cover	4		
Standard : 800 pg/ml	4 Vials Freeze Dried	Yellow	Add 1.08 ml of adequate standard buffer diluent to 1 vial .
Control : 188+/- 40 pg/ml	4 Vials Freeze Dried	Silver	Add 1.0 ml of adequate standard diluent to 1 vial
Standard Buffer Diluent	1 Vial (25 ml)	Black	Dilute in distilled Water
Standard Buffer : Human Serum	2 Vials (7 ml)	Black	Ready-to-use
Biotinylated anti- TNF α	2 Vials (0.3 ml)	Red	Dilute in biotinylated antibody diluent
Biotinylated Antibody Diluent	1 Vial (13 ml)	Red	Ready-to-use
Streptavidin-HRP	4 Vials (5 μ l)		0.5 ml of HRP-Diluent before further dilutions
HRP Diluent	1 Vial (23 ml)	Red	Ready-to-use
Washing Buffer	2 Vials (10 ml)	White	Dilute in distilled Water
Chromogen TMB :	1 Vial (21 ml)		Ready-to-use
H2SO4 : Stop Reagent	2 Vials (11 ml)	Black	Ready-to-use

* This is a generic data sheet and may be subject to change. Please contact Cell Sciences for the latest updated version.



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SAFETY

- * For research use only.
- * The human blood components included in this kit have been tested and found non reactive for HBsAg and anti-HIV. Nevertheless, no known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures , e.g. CDC/NIH Health manual : " Biosafety in Microbiological and Biomedical Laboratories" 1984.
 - * Avoid any skin contact with H₂SO₄ and TMB. In case of contact, wash thoroughly with water.
 - * Do not eat, drink, smoke or apply cosmetics where kit reagents are used.
 - * Do not pipette with mouth

PROCEDURAL NOTES/LAB. QUALITY CONTROL

1. When not in use, kit components should be stored refrigerated or frozen as indicated on vials or bottles labels. All reagents should be warmed to room temperature before use. Lyophilized standards and controls should be discarded right after resuspension and use.
2. Once the desired number of strips has been removed, immediately reseal the bag to protect the remaining strips from deterioration.
3. Cover or cap all reagents when not in use.
4. Do not mix or interchange reagents between different kit lots.
5. Do not use reagents beyond the expiration date of the kit.
6. Use a clean disposable plastic pipette tip for each reagent, standard, control or specimen addition in order to avoid cross-contamination ; for the dispensing of H₂SO₄ and substrate solution, avoid pipettes with metal parts.
7. Use a clean plastic container to prepare the washing solution.
8. Thoroughly mix the reagents and samples before use by agitation or swirling.
9. control should be run with each assay. If control values read outside pre-established ranges, the accuracy of the assay is suspect.
10. All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
11. The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent colour development. Warning TMB is toxic avoid direct contact with hands. Dispose off properly.
12. If a dark blue colour develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded. Read absorbances within 1 hour after completion of the assay.
13. Serum and plasma samples should be collected in pyrogen/endotoxin-free tubes.
14. Samples should be stored frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen specimens. Thaw completely prior to analysis.
15. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particles are present, this should be removed prior to assay by centrifugation or filtration.
16. When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells.
17. Respect incubation times described in the assay procedure.
18. Dispense the TMB solution within 15 min. following the washing of the microtiter plate.



REAGENTS PREPARATIONS

Standard buffer diluent

Dilute 5 ml of standard buffer diluent in 50 ml of distilled water or all the content of the vial (25 ml) in 250 ml of distilled water.

Standards

Two standards diluents are provided. For culture supernatants samples use standard buffer diluent with BSA (Standard Buffer Diluent). For sera or plasma samples use standard diluent with human serum (Standard Diluent : human serum). Standard have to be reconstituted with 1.08 ml of adequate standard buffer diluent. Controls have to be reconstituted with 1.0 ml of adequate standard diluent.

Dilution of biotinylated anti- TNF α

Dilute the biotinylated anti- TNF α with the biotinylated antibody diluent in a clean glass vial according to the number of wells to be used. See the next table for volumes to pipette. Extemporaneous preparations are recommended.

Number of Wells used	Biotinylated Antibody (μl)	Biotinylated Antibody Diluent (μl)
16	40	1060
24	60	1590
32	80	2120
48	120	3180
96	240	6360

Dilution of Streptavidin-HRP

Add 0.5 ml of HRP diluent to a 5 μ l vial of Streptavidin-HRP . DO NOT KEEP THIS DILUTION FOR FURTHER EXPERIMENTS. Extemporaneous preparations are recommended. Following the number of wells to be used, further dilutions of Streptavidin-HRP should be made with HRP diluent in a clean glass vial : see hereafter the table for volumes to pipette.

Number of Wells	Streptavidin-HRP(μl)	Strep-HRP Diluent (ml)
16	30	2
24	45	3
32	60	4
48	75	5
96	150	10

Dilution of Washing Buffer

Dilute 2 ml of washing buffer in 400 ml of distilled water or all the content of one vial (10 ml) in 2000 ml of distilled water.



ASSAY METHOD

- a) Before use, mix all reagents thoroughly without making foam.
- b) Determine the number of microwell strips required to test the desired number of samples, plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control samples should be assayed in duplicate. Remove sufficient microwell strips from the pouch.
- c) Add 100 µl of appropriate standard diluent to standard wells B1, B2, C1, C2, D1, D2, E1, E2, F1, F2. Reconstitute standard vial with the appropriate volume as described in the chapter reagents preparation. Pipet 200 µl of standard into wells A1 and A2 (see Plate Scheme below). Transfer 100 µl from A1 and A2 to B1 and B2 wells. Mix the contents by repeated aspirations and ejections. Take care not to scratch the inner surface of microwells. Repeat this procedure from the wells B1, B2 to wells C1, C2 and from wells C1, C2 to D1, D2 and so on creating two parallel rows of TNF α standard dilutions ranging from 800 to 25 pg/ml. Discard 100 µl from the content of the last microwells used (F1, F2).
- d) Add 100 µl of appropriate standard diluent to the blank wells (G1-G2).
- e) Add 100 µl of sample to sample wells and 100µl of control to control wells (H1, H2)
- f) Preparation of biotinylated anti- TNF α : see reagents preparation.
- g) Add 50 µl of diluted biotinylated anti- TNF α to all wells.
- h) Cover with a plate cover and incubate for 3 hours at room temperature (18°C - 25°C).
- i) Remove the cover plate and wash the plate as follows:
 - 1) aspirate the liquid from each well ;
 - 2) dispense 0.3 ml of washing solution into each well ;
 - 3) aspirate again the content of each well ;
 - 4) Repeat steps 2) and 3) two times.
- j) Prepare HRP solution just before use: see reagents preparation.
- k) Dispense 100 µl of HRP solution into all wells, including the blank wells. Put back the cover plate.
- l) Incubate the microwell strips at room temperature for 30 minutes.
- m) Remove plate cover and empty wells. Wash microwell strips according to point (i). Proceed immediately to the next step.
- n) Pipette 100 µl of ready-to-use TMB substrate solution into all wells, including the blank wells and incubate in the dark for 12-15 minutes at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil.
- o) Incubation time of the substrate solution is usually determined by the ELISA reader performances: many ELISA readers record absorbance only up to 2.0 O.D. The O.D. values of the plate should be monitored and the substrate reaction stopped before positive wells are no longer properly readable (maximum 20 minutes).
- p) The enzyme-substrate reaction is stopped by quickly pipetting 100 µl of 1.8 N sulfuric acid into each well, including the blank wells, to completely and uniformly inactivate the enzyme. Results must be read immediately after the addition of sulfuric acid, or within one hour, if the microwell strips are stored at 2-8°C in the dark.
- q) Read absorbance of each well on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm (610 nm to 650 nm is acceptable) as the reference wavelength.

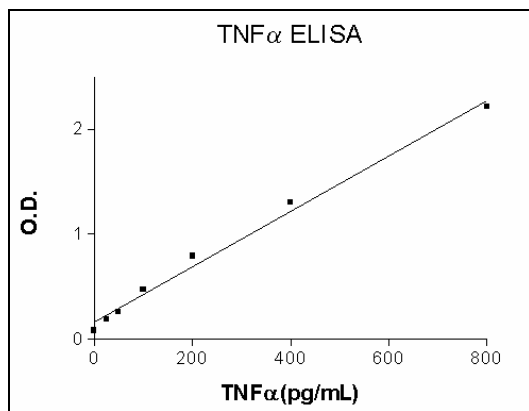


SUGGESTED PLATE SCHEME

	Standard Concentrations pg/mL		Sample wells									
	1	2	3	4	5	6	7	8	9	10	11	12
A	800	800										
B	400	400										
C	200	200										
D	100	100										
E	50	50										
F	25	25										
G	Blank	Blank										
H	Ctrl	Ctrl										

DATA ANALYSIS

Generate a linear standard curve by plotting the average absorbance on the vertical axis versus the corresponding TNF α standard concentration on the horizontal axis. The amount of TNF α in each sample is determined by extrapolating OD values to TNF α concentrations using the standard curve.



Typical TNF α standard curve ranging from 25 to 800pg/mL



LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the 800 pg/ml standard curve point. The dose-response is non-linear in this region and good accuracy is difficult to obtain. Concentrated samples (> 800 pg/ml) have to be diluted with standard diluent or with your own sample buffer. During analysis, multiply results by the appropriate dilution factor.

The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced...) and the use of plasma instead of serum specimens has not been thoroughly investigated. The rate of degradation of native TNF α in various matrices has not been investigated. The immunoassay literature contains frequent references to aberrant signals observed with some human sera and attributed to heterophilic (or anti-mouse) antibodies. Though we have not found such specimens to date, the possibility of this occurrence can not be excluded.

PERFORMANCES AND CHARACTERISTICS

Sensitivity

The minimum detectable dose of TNF α is less than 10 pg/ml.

This has been determined by adding 3 standard deviations to the mean optical density obtained when the zero standard was assayed 38 times.

Precision

Intra-Assay					Inter-Assay				
Sample	n	Mean (pg/mL)	SD	CV%	Sample	n	Mean (pg/mL)	SD	CV%
A	8	794.8	6.823	0.85	A	89	797.6	13.63	1.70
B	8	100.6	3.121	3.09	B	89	101.9	6.123	6.00

Linearity of dilution

A human serum pool containing 800 pg/ml of measured TNF α was serially diluted in standard buffer diluent over the range of the assay. Linear regression of samples versus the expected concentration yielded a correlation coefficient of 0.99.

Recovery

Recovery of TNF α added to pooled normal serum was 95.5% (82.9% to 100%) for TNF α concentration ranging from 800 to 25 pg/ml.



ASSAY PROCEDURE SUMMARY Total procedure length : 3h45

