Human TNF-alpha Sandwich ELISA Kit Datasheet

For the quantitative detection of human TNF-alpha concentrations in serum, plasma and cell culture supernatants.

General Information

Catalogue Number	KE00068
Product Name	Human TNF-alpha Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	31.25-2000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	7124
SwissProt	P01375

Kit Components & Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	**Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.**
Protein standard - 4000 pg/bottle; lyophilized*	2 bottles	
Detection antibody, biotinylated (100X) - 120 μL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 μL/vial	1 vial	
Sample Diluent PT 1-e - 30 mL/bottle. For serum and plasma samples	1 bottle	
Sample Diluent PT 1-ef - 30 mL/bottle. For cell culture supernatants	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	3 pieces	

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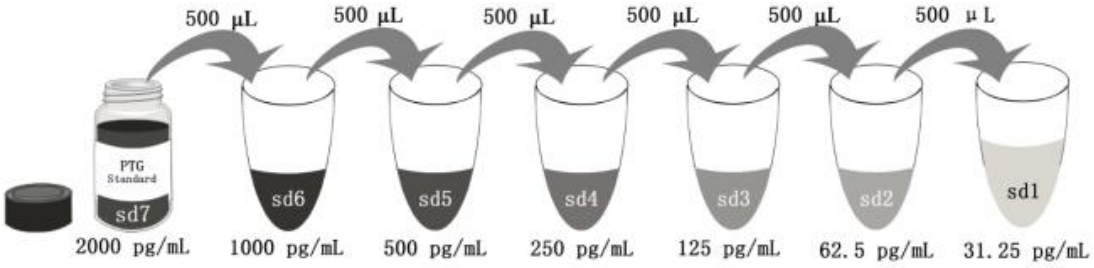
NB: Do not use the kit after the expiration date.
Sample Diluent PT 1-e is for protein standard, serum and plasma samples.
Sample Diluent PT 1-ef is for protein standard and cell culture supernatants.
Detection Diluent is for Detection antibody and Streptavidin-HRP.
*Add **2 mL** Sample Diluent PT 1-e or PT 1-ef in protein standard. This reconstitution gives a stock solution of 2000 pg/mL.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 1-e or PT 1-ef	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00068 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The TNF-α ELISA kit is to be used to detect and quantify protein levels of endogenous TNF-α. The assay recognizes human TNF-α. An antibody specific for TNF-α has been pre-coated onto the microwells. The TNF-α protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for TNF-α is added to detect the captured TNF-α protein. For signal development, Streptavidin-HRP is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm.

Background

TNF, as also known as TNF-alpha, or cachectin, is a multifunctional proinflammatory cytokine that belongs to the tumor necrosis factor (TNF) superfamily. It is expressed as a 26 kDa membrane bound protein and is then cleaved by TNF-alpha converting enzyme (TACE) to release the soluble 17 kDa monomer, which forms homotrimers in circulation. It is produced chiefly by activated macrophages, although it can be produced by many other cell types such as CD4+ lymphocytes, NK cells, neutrophils, mast cells, eosinophils, and neurons. It can bind to, and thus functions through its receptors TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR. This cytokine is involved in the regulation of a wide spectrum of biological processes including cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation. Dysregulation of TNF production has been implicated in a variety of human diseases including Alzheimer's disease, cancer, major depression and inflammatory bowel disease (IBD).

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Sample Preparation

The serum or plasma samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:2, 1:4 is suggested according to the individual samples.

Safety Notes

This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 µL	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

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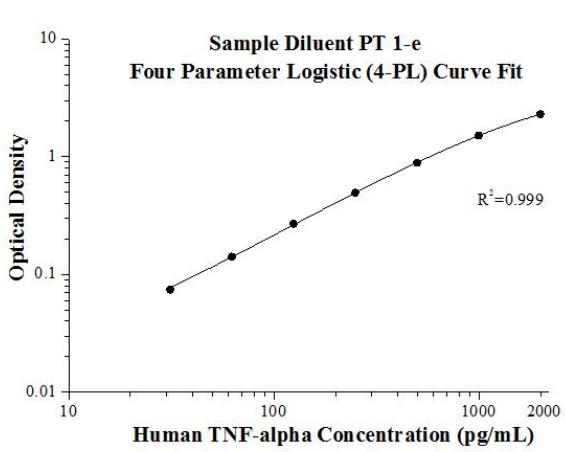
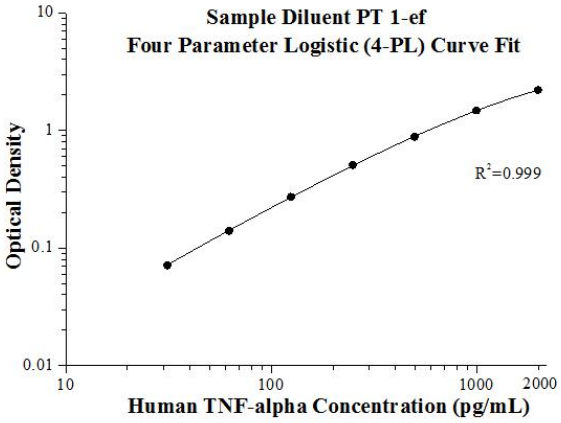
Example data

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D	Average	Corrected
0	0.078 0.074	0.076	-
31.25	0.157 0.143	0.15	0.074
62.5	0.23 0.204	0.217	0.141
125	0.356 0.331	0.344	0.268
250	0.589 0.546	0.568	0.492
500	0.981 0.942	0.962	0.886
1000	1.616 1.552	1.584	1.508
2000	2.398 2.339	2.369	2.293



(pg/mL)	O.D	Average	Corrected
0	0.072 0.072	0.072	-
31.25	0.142 0.144	0.143	0.0071
62.5	0.213 0.21	0.212	0.140
125	0.332 0.353	0.343	0.271
250	0.571 0.578	0.579	0.507
500	0.938 0.966	0.952	0.880
1000	1.536 1.555	1.546	1.474
2000	2.273 2.274	2.274	2.202

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Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	20	365.8	11.0	3.0
2	20	747.8	42.2	5.6
3	20	1,508.1	96.9	6.4

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	392.2	21.0	5.4
2	24	774.4	33.5	4.3
3	24	1,572.5	61.9	3.9

Recovery

The recovery of TNF-alpha spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated. (The plasma sample were initially diluted 1:1)

Sample Type		Average% of Expected	Range (%)
Human plasma	1:2	75	70-78
	1:4	78	72-86
Cell culture supernatants	1:2	105	81-126
	1:4	95	84-106

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Sample Values

Twenty-four serum and plasma samples from healthy volunteers were evaluated for human TNF-alpha in this assay. All samples measured less than the lowest standard, 15.6 pg/mL. No medical histories were available for the donors used in this study.

Cell Culture supernatants - Human peripheral blood mononuclear cells (PBMC) (5×10^5 cells/mL) were cultured in RPMI-1640 supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin sulfate. The cell culture supernatants were stimulated for different conditions and assayed for human TNF-alpha. (*** Day 1 : PBMC were stimulated used by 10 ug/mL PHA 1 day and 50 ng/mL LPS 2 hours; Day 3: PBMC were stimulated used by 10ug/mL PHA 2 days and 50ng/mL LPS 1 day; Day 5: PBMC were stimulated used by 10 ug/mL PHA 3 days and 50ng/mL LPS 2 days**)

Stimulated conditions	Day 1 (pg/mL)	Day 3 (pg/mL)	Day 5 (pg/mL)
PHA 10ug/mL	1,025	2,192	1,424
PHA 10ug/mL+ LPS 50ng/mL *	1,880	2,383	1,143
Unstimulated	349	254	150

Sensitivity

The minimum detectable dose of human TNF-alpha is 1.0 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

Linearity

To assess the linearity of the assay, three samples were spiked with high concentrations of TNF-alpha in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

		Human plasma	Cell culture supernatants
1:2	Average% of Expected	74	91
	Range (%)	73-78	88-94
1:4	Average% of Expected	82	98
	Range (%)	76-87	95-102
1:8	Average% of Expected	87	98
	Range (%)	75-92	94-101
1:16	Average% of Expected	90	98
	Range (%)	75-104	97-98

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References

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