Mouse TNF-alpha Sandwich ELISA Kit Datasheet

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

General Information

Catalogue Number	KE10002
Product Name	Mouse TNF-alpha Sandwich ELISA Kit
Species cross-reactivity	Mouse
Range (calibration Range)	7.8-500 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	21926 (Mouse)
SwissProt	P06804 (Mouse)

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 - 1000 pg/bottle; lyophilized*	2 bottles	
Detection antibody, biotinylated (100×) - 120 µL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100×) - 120 µL/vial	1 vial	
Sample Diluent PT 1-ef - 30 mL/bottle.	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 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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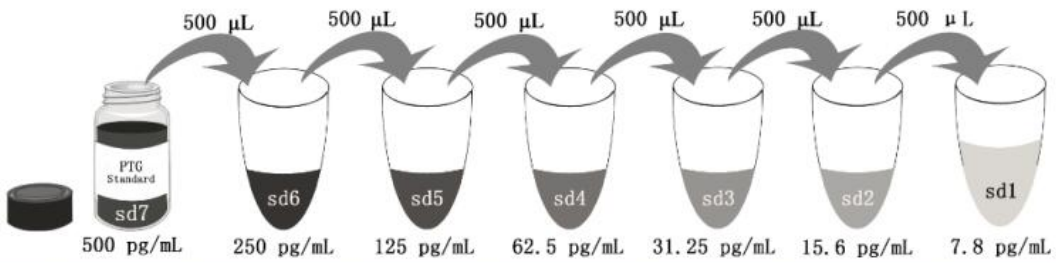
NB: Do not use the kit after the expiration date.
Sample Diluent PT 1-ef is for protein standard and samples.
Detection Diluent is for Detection antibody and Streptavidin-HRP.
*Add **2 mL** Sample Diluent PT 1-ef in protein standard. This reconstitution gives a stock solution of 500 pg/mL.



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-ef	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE10002 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The TNF-alpha ELISA kit is to be used to detect and quantify protein levels of endogenous TNF-alpha. The assay recognizes mouse TNF-alpha. An antibody specific for TNF-alpha has been pre-coated onto the microwells. The TNF-alpha protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for TNF-alpha is added to detect the captured TNF-alpha 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. Mouse and human TNF-alpha share 79% amino acid sequence identity. Unlike human TNF-alpha, the mouse form is glycosylated. In mouse deficiency of this gene is associated with defects in response to bacterial infection, with defects in forming organized follicular dendritic cell networks and germinal centers, and with a lack of primary B cell follicles.

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Sample Preparation

The plasma and cell culture supernatants 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

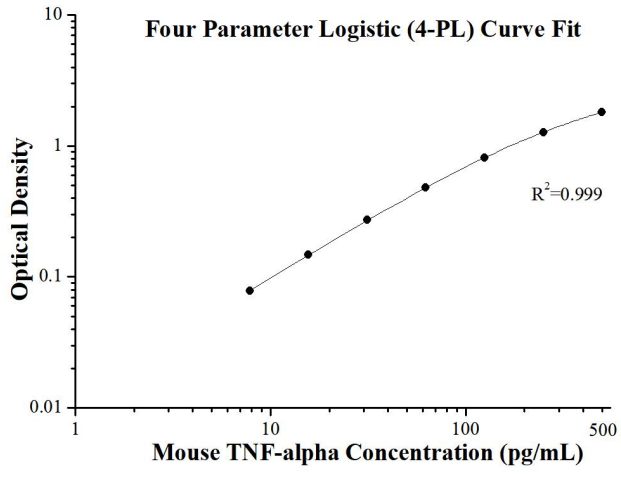
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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.				

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	0.067 0.063	0.065	-
7.8	0.151 0.134	0.143	0.078
15.6	0.218 0.207	0.213	0.148
31.25	0.347 0.325	0.336	0.271
62.5	0.543 0.549	0.546	0.481
125	0.899 0.856	0.878	0.813
250	1.397 1.286	1.342	1.277
500	1.912 1.842	1.877	1.812

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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	72.4	3.3	4.5
2	20	154.8	11.1	7.2
3	20	295.8	19.7	6.7

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	58.9	4.4	7.5
2	24	128.0	6.8	5.3
3	24	248.0	17.4	7.0

Recovery

The recovery of TNF-alpha spiked to three different levels in four samples throughout the range of the assay in serum and cell culture supernatants was evaluated. (The serum was initially diluted 1:2)

Sample Type		Average% of Expected	Range (%)
Mouse serum	1:2	73	63-83
	1:4	91	83-99
Cell culture supernatants	1:2	95	85-101
	1:4	92	89-104

Sample Values

RAW 264.7 mouse monocyte/macrophage cells (3.5 x 10^6cells/mL) were cultured in DMEM supplemented with 8% fetal bovine serum, 2.5 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 0.1 µg/mL LPS for 1 day or 3 days. Aliquots of the cell culture supernates were removed and assayed for levels of mouse TNF-alpha.

Condition	(pg/mL)
Unstimulated	11.0
Stimulated for 1d	593.0
Stimulated for 3d	170.0

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Sensitivity

The minimum detectable dose of mouse 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 serum and cell culture supernatants and diluted with **Sample Diluent PT 1-ef** to produce samples with values within the dynamic range of the assay. (The sample was initially diluted 1:2)

		Mouse serum	Cell culture supernatants
1:2	Average% of Expected	60	86
	Range (%)	57-63	84-87
1:4	Average% of Expected	76	87
	Range (%)	71-81	81-93
1:8	Average% of Expected	89	92
	Range (%)	86-89	89-94
1:16	Average% of Expected	108	90
	Range (%)	106-110	88-91

References

1. Agbanoma G. et al. (2012) J Immunol. 188: 1307-17.
2. Kriegler M. et al. (1988) Cell. 53: 45-53.
3. Theiss AL. et al. (2005) J Biol Chem. 280: 36099-109.
4. Swardfager W. et al. (2010) Biol Psychiatry. 68:930-41.
5. Locksley RM.et al. (2001) Cell. 104(4):487-501.
6. provided by RefSeq, Jun 2013

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