Mouse TNFR1 Sandwich ELISA Kit Datasheet

For the quantitative detection of mouse TNFR1 in serum, plasma and cell culture supernatants.

General Information

Catalogue Number	KE10065
Product Name	Mouse TNFR1 Sandwich ELISA Kit
Species cross-reactivity	Mouse
Range (calibration Range)	7.8-500 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	21937
SwissProt	P25118

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 - 500 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 3 - 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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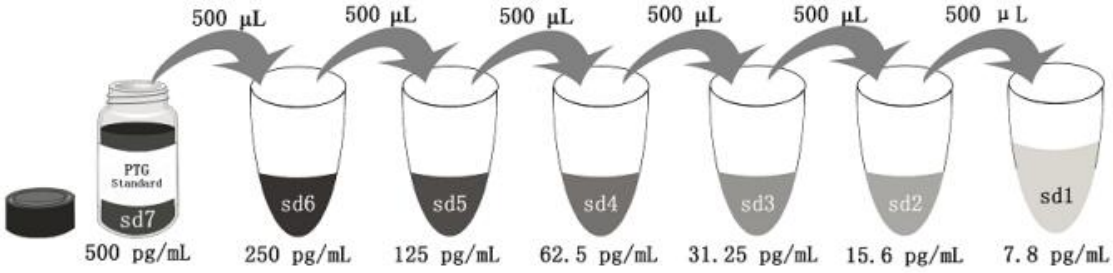
NB: Do not use the kit after the expiration date.
Sample Diluent PT 3 is for protein standard and samples.
Detection Diluent is for Detection antibody and Streptavidin-HRP.
*Add **1 mL** Sample Diluent PT 3 in protein standard. This reconstitution gives a stock solution of 500 pg/mL.



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 3	**1000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE10065 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The MouseTNFR1 ELISA kit is to be used to detect and quantify protein levels of endogenous mouseTNFR1. The assay recognizes mouseTNFR1. An antibody specific for mouseTNFR1 has been pre-coated onto the microwells. The mouseTNFR1 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for mouseTNFR1 is added to detect the captured mouseTNFR1 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

Tumor necrosis factor (TNF) is a multifunctional cytokine that plays a key role in regulating inflammation, immune functions, host defense, and apoptosis. TNF signals through two distinct cell surface receptors, TNFR1 (TNFRSF1A, CD120a) and TNFR2 (TNFRSF1B, CD120b). TNFR1, which contains a death domain (DD) within its intracytoplasmic region, is thought to be the key receptor for TNF signaling. This receptor can be released to the extracellular space by two mechanisms, ectodomain shedding and constitutive release of full-length 55-kDa TNFR1. Soluble TNFR1 (sTNFR1) could function as TNF-binding protein, inhibiting TNF bioactivity.

Sample Preparation

Different samples may require proper dilution to fall within the range of the assay. The mouse serum is better to be diluted 1:4 or

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1:8 before assay, 1:2 or 1:4 dilution is recommended for cell culture supernatants.

Safety Notes

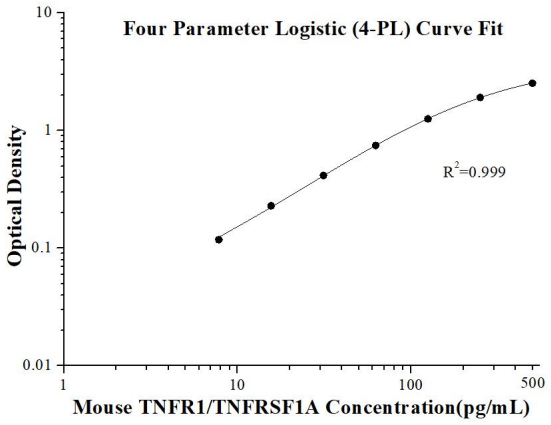
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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	0.026 0.027	0.027	-
7.8	0.145 0.144	0.145	0.118
15.6	0.256 0.255	0.256	0.229
31.25	0.435 0447	0.441	0.4145
62.5	0.769 0.777	0.773	0.7465
125	1.257 1.305	1.281	1.2545
250	1.922 1.951	1.937	1.91
500	2.519 2.583	2.551	2.551

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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	219.9	6.3	2.9
2	20	52.2	1.3	2.5
3	20	12.1	0.4	3.0

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	205.8	7.4	3.6
2	24	51.9	2.1	4.0
3	24	12.5	0.6	4.7

Recovery

The recovery of TNFR1 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Mouse serum	1:15	99	81-123
	1:30	96	80-114
Cell culture supernatants	1:8	100	78-124
	1:16	91	71-124

Sample Values

Mouse serum samples were evaluated for the presence of mouse TNFR1 in this assay.

Sample Type	Mean of Detectable (pg/mL)	Range (pg/mL)
Mouse serum (n=16)	717.6	455.7-949.9

L-929 mouse fibroblast cells (1 x 10^6 cells/mL) were cultured in MEM containing L-glutamine and 10% equine serum for 3 days. The cell culture supernate was removed, assayed for mouse TNFR1, and measured 174.1 pg/mL.

Sensitivity

The minimum detectable dose of mouse TNFR1 is 0.5 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

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Linearity

To assess the linearity of the assay, samples were diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.

(The serum samples were initially diluted 1:2)

		Mouse serum	Cell culture supernatants
1:2	Average% of Expected	100	100
	Range (%)	-	-
1:4	Average% of Expected	103	104
	Range (%)	97-110	96-118
1:8	Average% of Expected	112	114
	Range (%)	111-114	113-115
1:16	Average% of Expected	103	108
	Range (%)	99-107	97-115

References

1. Islam A, et al. Extracellular TNFR1 release requires the calcium-dependent formation of a nucleobindin 2-ARTS-1 complex. J Biol Chem. 281(10):6860-73 (2006).
2. Aggarwal BB, et al. Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey. Blood. 119(3):651-65 (2012).
3. Aderka D , et al. Stabilization of the bioactivity of tumor necrosis factor by its soluble receptors. J Exp Med. 175(2):323-9 (1992).

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