

## Mouse TNFRSF1B Sandwich ELISA Kit Datasheet

For the quantitative detection of Mouse TNFRSF1B in serum, plasma and cell culture supernatants.

### General Information

Catalogue Number	KE10061
Product Name	Mouse TNFRSF1B Sandwich ELISA Kit
Species cross-reactivity	Mouse
Range (calibration Range)	3.9-250 pg/mL
Tested applications	Quantification ELISA

### Database Links

Entrez Gene	21938
SwissProt	P25119

### Kit Components & Storage

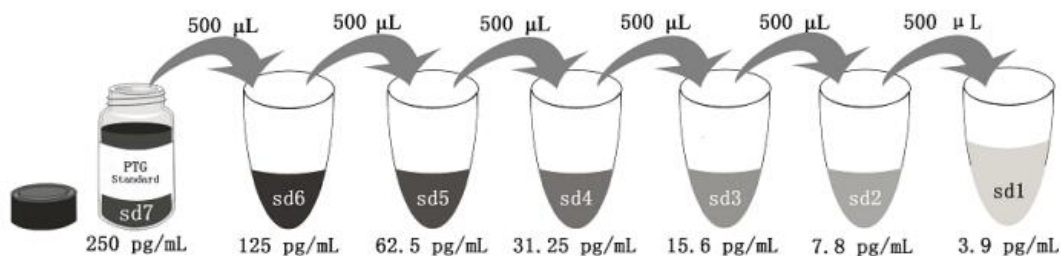
Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	<b>Unopened Kit:</b> Store at 2-8°C for 6 months or -20°C for 12 months.  <b>Opened Kit:</b> All reagents stored at 2-8°C for 7 days.  <b>Please use a new standard for each assay.</b>
Protein standard - 250 pg/bottle; lyophilized*	2 bottles	
Detection antibody, biotinylated (100X) - 120 µL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 4 - 30 mL/bottle.	2 bottles	
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	

**NB: Do not use the kit after the expiration date.**

Sample Diluent PT 4 is for protein standard and samples.

Detection Diluent is for Detection antibody and Streptavidin-HRP.

\*Add 1 mL Sample Diluent PT 4 in protein standard. This reconstitution gives a stock solution of 250 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 4	<b>1000 µL</b>	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

## Product Description

KE10061 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The TNFRSF1B ELISA kit is to be used to detect and quantify protein levels of endogenous TNFRSF1B. The assay recognizes mouse TNFRSF1B. An antibody specific for TNFRSF1B has been pre-coated onto the microwells. The TNFRSF1B protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for mouse TNFRSF1B is added to detect the captured mouse TNFRSF1B 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

TNFRSF1B, also known as TNFR2 or CD120b or p75, is a member of the tumor necrosis factor receptor superfamily, which also contains TNFRSF1A. Tumor necrosis factor (TNF, TNFSF2) is a multifunctional cytokine that plays a key role in regulating inflammation, immune functions, host defense, and apoptosis. TNF signals through TNFRSF1A and TNFRSF1B. TNFRSF1A is widely expressed, whereas TNFRSF1B exhibits more restricted expression, being found on CD4 and CD8 T lymphocytes, endothelial cells, microglia, oligodendrocytes, neuron subtypes, cardiac myocytes, thymocytes and human mesenchymal stem cells. Both TNFRSF1A and TNFRSF1B can also exist in soluble forms, probably derived by proteolytic cleavage from the cell surface forms. Various defects in the TNFRSF1B pathway, due to polymorphisms in the TNFRSF1B gene, upregulated expression of TNFRSF1B and TNFRSF1B shedding, have been implicated in the pathology of several autoimmune disorders.

## Sample Preparation

Samples may require proper dilution to fall within the range of the assay. 1:80 or 1:160 dilution is recommended for serum or

plasma. Cell culture supernatants is better to be diluted 1:8 or 1:16.

## Safety Notes

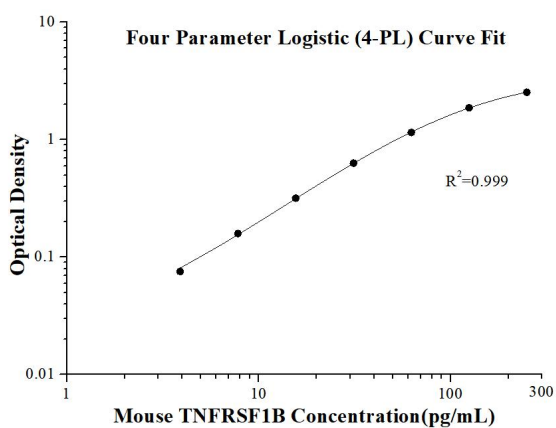
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

## 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	0.028 0.026	0.027	-
3.9	0.096 0.109	0.103	0.076
7.8	0.184 0.188	0.186	0.159
15.6	0.342 0.347	0.345	0.318
31.25	0.645 0.671	0.658	0.631
62.5	1.179 1.177	1.178	1.151
125	1.897 1.888	1.893	1.866
250	2.510 2.603	2.557	2.53

## 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					Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%	Sample	n	Mean (pg/mL)	SD	CV%
1	20	108.5	3.9	3.6	1	24	92.9	8.6	9.3
2	20	33.3	0.7	2.0	2	24	30.7	2.4	7.9
3	20	15.6	0.5	3.2	3	24	14.5	1.3	9.3

## Recovery

The recovery of TNFRSF1B 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	1:300	111	99-123
	1:600	107	103-112
Cell culture supernatants	1:30	120	111-128
	1:60	115	109-122

## Sample Values

Mouse serum samples were evaluated for the presence of mouse TNFRSF1B in this assay.

Sample Type	Mean of Detectable (pg/mL)	Range (pg/mL)
mouse serum (n=16)	6,345.5	5,311.9-7,676.5

Mouse lung conditioned media cultured in RPMI supplemented with 10% fetal bovine serum was collected after culturing for 5 days. An aliquot of the cell culture supernate was removed, assayed for mouse TNFRSF1B and measured 596.6 pg/mL.

## Sensitivity

The minimum detectable dose of mouse TNFRSF1B is 0.1 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, 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 1:40. The cell culture supernatants were initially diluted 1:4 )

		mouse serum	Cell culture supernatants
1:2	Average% of Expected	100	100
	Range (%)	-	-
1:4	Average% of Expected	104	102
	Range (%)	99-107	98-107
1:8	Average% of Expected	104	102
	Range (%)	101-110	95-112
1:16	Average% of Expected	104	98
	Range (%)	99-107	93-106

## References

1. Islam A, Adamik B, Hawari FI, et al. Extracellular TNFR1 release requires the calcium-dependent formation of a nucleobindin 2-ARTS-1 complex. *J Biol Chem.* 2006;281(10):6860-6873.
2. Faustman D, Davis M. TNF receptor 2 pathway: drug target for autoimmune diseases. *Nat Rev Drug Discov.* 2010;9(6):482-493.
3. Cabal-Hierro L, Lazo PS. Signal transduction by tumor necrosis factor receptors. *Cell Signal.* 2012;24(6):1297-1305.
4. Diez-Ruiz A, Tilz GP, Zangerle R, Baier-Bitterlich G, Wachter H, Fuchs D. Soluble receptors for tumour necrosis factor in clinical laboratory diagnosis. *Eur J Haematol.* 1995;54(1):1-8.