

Human TNFRSF9 Sandwich ELISA Kit Datasheet

For the quantitative detection of Human TNFRSF9 concentrations in serum, plasma and cell culture supernatants.

General Information

Catalogue Number	KE00125
Product Name	Human TNFRSF9 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	7.8-500 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	3604
SwissProt	Q07011

Kit Components & Storage

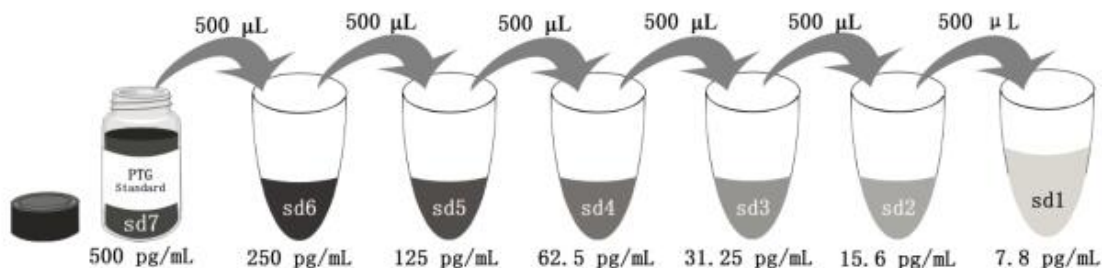
Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	Unopened Kit: Store at 2-8°C for 6 months or -20°C for 12 months. Opened Kit: All reagents stored at 2-8°C for 7 days. Please use a new standard for each assay.
Protein standard - 500 pg/bottle; lyophilized*	2 bottles	
Detection antibody, biotinylated (100X) - 120 µL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 3-ef - 30 mL/bottle	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	

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

Sample Diluent PT 3-ef is for protein standard and samples.

Detection Diluent is for Detection antibody and Streptavidin-HRP.

*Add 1 mL Sample Diluent PT 3-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 3-ef	1000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00125 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The TNFRSF9 ELISA kit is to be used to detect and quantify protein levels of endogenous TNFRSF9. The assay recognizes human TNFRSF9. An antibody specific for TNFRSF9 has been pre-coated onto the microwells. The TNFRSF9 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for TNFRSF9 is added to detect the captured TNFRSF9 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

CD137, also known as TNFRSF9 or 4-1BB, is an inducible T cell surface receptor which belongs to the tumor necrosis factor receptor superfamily. CD137 is a transmembrane protein expressed on the surface of activated T-cells. In addition, activation-dependent expression of CD137 has also been found in B lymphocytes, monocytes, and diverse nonlymphoid cell types. CD137 provides a co-stimulatory signal that enhances the survival, and differentiation of cells, and has a crucial role in the development of CD8 cytotoxic T cells and anti-tumor immunity. Soluble forms of CD137 (sCD137) are generated by differential splicing and are released by activated lymphocytes. Elevated serum sCD137 level has been associated with some diseases, including rheumatoid arthritis, multiple sclerosis, chronic lymphocytic leukemia, and acute coronary syndrome.

Sample Preparation

The serum or plasma, cell culture supernatants may require proper dilution to fall within the range of the assay. 1:4 or 1:8 dilution is recommended for serum or plasma. 1:2 or 1:4 dilution is recommended for cell culture supernatants.

Safety Notes

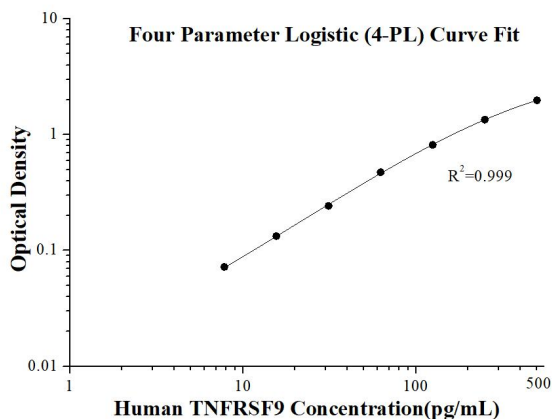
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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	0.092 0.091	0.092	-
7.8	0.162 0.165	0.164	0.072
15.6	0.224 0.225	0.225	0.133
31.25	0.327 0.341	0.334	0.243
62.5	0.556 0.574	0.565	0.474
125	0.901 0.911	0.906	0.815
250	1.438 1.441	1.440	1.348
500	2.071 2.071	2.071	1.980

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	241.1	7.1	2.9	1	24	246.6	14.5	5.9
2	20	60.3	1.1	1.8	2	24	59.2	2.4	4.1
3	20	13.9	0.8	5.8	3	24	14.4	0.7	4.9

Recovery

The recovery of TNFRSF9 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 (%)
Human serum	1:4	101	84-122
	1:8	92	77-104
Cell culture supernatants	1:2	95	89-100
	1:4	98	92-106

Sample Values

Human serum, plasma samples from healthy volunteers were evaluated for human TNFRSF9 in this assay.

Sample Type	Mean(pg/mL)	Rang (pg/mL)
Human plasma (n=12)	127.0	33.1-392.6
Human serum (n=12)	224.9	44.8-682.1

Cell culture supernates-Human peripheral blood mononuclear cells (1×10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, $50 \mu\text{M}$ β -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin and $100 \mu\text{g/mL}$ streptomycin sulfate. Cells were stimulated with 10 $\mu\text{g/mL}$ PHA. Aliquots of the cell culture supernate were removed on days 3 and assayed for levels of human TNFRSF9.

Condition	Day 3 (pg/mL)
Unstimulated	60
Stimulated	428

Sensitivity

The minimum detectable dose of human TNFRSF9 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, cell culture supernatants samples were spiked with high concentrations of human TNFRSF9 in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. Serum were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

		Human serum	Cell culture supernatants
1:2	Average% of Expected	100	104
	Range (%)	95-106	100-107
1:4	Average% of Expected	113	104
	Range (%)	100-124	96-107
1:8	Average% of Expected	103	103
	Range (%)	88-120	96-109
1:16	Average% of Expected	91	105
	Range (%)	75-107	99-110

References

1. Vinay DS, et al. Role of 4-1BB in immune responses. *Semin Immunol.* 10(6):481-9 (1998).
2. Schwarz H, et al. ILA, the human 4-1BB homologue, is inducible in lymphoid and other cell lineages. *Blood.* 85(4):1043-52 (1995).
3. Nakaima Y, et al. CD137 is induced by the CD40 signal on chronic lymphocytic leukemia B cells and transduces the survival signal via NF- κ B activation. *PLoS One.* 16;8(5):e64425 (2013).
4. Michel J, et al. A soluble form of CD137 (ILA/4-1BB), a member of the TNF receptor family, is released by activated lymphocytes and is detectable in sera of patients with rheumatoid arthritis. *Eur J Immunol.* 28(1):290-5 (1998).
5. Sharief MK, et al. Heightened intrathecal release of soluble CD137 in patients with multiple sclerosis. *Eur J Neurol.* 9(1):49-54 (2002).
6. Furtner M, et al. Levels of soluble CD137 are enhanced in sera of leukemia and lymphoma patients and are strongly associated with chronic lymphocytic leukemia. *Leukemia.* 19(5):883-5 (2005).
7. Yan J, et al. Clinical implications of elevated serum soluble CD137 levels in patients with acute coronary syndrome. *Clinics (Sao Paulo).* 68(2):193-8 (2013).