

Mouse Osteoprotegerin/TNFRSF11B Sandwich ELISA Kit Datasheet

For the quantitative detection of mouse Osteoprotegerin/TNFRSF11B in serum, plasma and cell culture supernatants samples.

General Information

Catalogue Number	KE10058
Product Name	Mouse Osteoprotegerin/TNFRSF11B Sandwich ELISA Kit
Species cross-reactivity	Mouse
Range (calibration Range)	31.3-2000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	18383
SwissProt	O08712

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 5 - 30 mL/bottle. For serum and plasma	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	

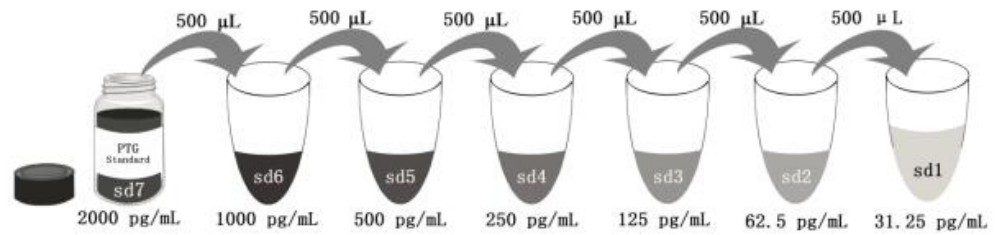
NB: Do not use the kit after the expiration date.

Sample Diluent PT 5 is for protein standard, serum and plasma samples.

Sample Diluent PT 1-ef is for protein standard and cell culture supernatants samples.

Detection Diluent is for Detection antibody and Streptavidin-HRP.

*Add 2 mL Sample Diluent PT 5 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 5 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

KE10058 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The mouse Osteoprotegerin/TNFRSF11B ELISA kit is to be used to detect and quantify protein levels of endogenous mouse Osteoprotegerin/TNFRSF11B. The assay recognizes mouse Osteoprotegerin/TNFRSF11B. An antibody specific for mouse Osteoprotegerin/TNFRSF11B has been pre-coated onto the microwells. The mouse Osteoprotegerin/TNFRSF11B protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for mouse Osteoprotegerin/TNFRSF11B is added to detect the captured mouse Osteoprotegerin/TNFRSF11B 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

Osteoprotegerin (OPG), also known as TNFRSF11B or OCIF, is a member of the tumor necrosis factor receptor super family (TNFRSF). It is a secreted glycoprotein that inhibits osteoclastogenesis and is involved in the regulation of bone density. Osteoprotegerin acts as decoy receptor for receptor activator of nuclear factor-kappa B ligand (RANKL) and neutralizes its function in osteoclastogenesis. Osteoprotegerin may function as a soluble decoy receptor for tumor necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL) and inhibits TRAIL-induced apoptosis. TRAIL blocks osteoprotegerin mediated inhibition of osteoclastogenesis.

Sample Preparation

Different samples may require proper dilution to fall within the range of the assay. The serum, plasma and cell culture supernatants is better to be diluted 1:2 or 1:4 before assay.

Safety Notes

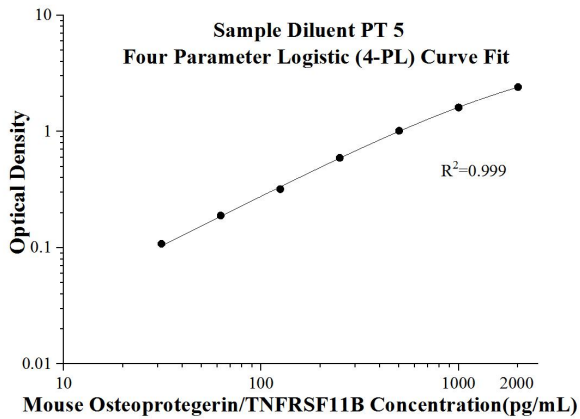
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

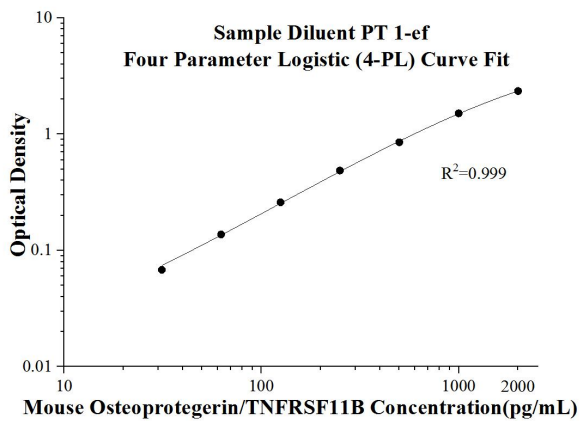
Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	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	0.071 0.068	0.070	-
31.3	0.182 0.173	0.178	0.108
62.5	0.263 0.255	0.259	0.189
125	0.399 0.378	0.389	0.319
250	0.675 0.651	0.663	0.593
500	1.123 1.047	1.085	1.015
1000	1.695 1.662	1.679	1.609
2000	2.531 2.424	2.478	2.408



(pg/mL)	O.D	Average	Corrected
0	0.073 0.069	0.071	-
31.3	0.14 0.138	0.139	0.068
62.5	0.209 0.207	0.208	0.137
125	0.341 0.319	0.330	0.259
250	0.553 0.562	0.558	0.487
500	0.925 0.917	0.921	0.85
1000	1.591 1.567	1.579	1.508
2000	2.426 2.405	2.416	2.345

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	48.0	1.7	3.5	1	24	46.6	1.8	3.9
2	20	207.5	4.9	2.3	2	24	195.6	9.3	4.7
3	20	859.6	25.6	3.0	3	24	806.4	36.0	4.5

Recovery

The recovery of Osteoprotegerin/TNFRSF11B 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	1:8	97	73-122
	1:16	102	96-121
Cell culture supernates	1:2	102	73-114
	1:4	95	80-106

Sample Values

Serum/Plasma

Sixteen individual mouse serum samples were evaluated for the presence of mouse Osteoprotegerin/TNFRSF11B in this assay.

Sample Type	Mean (pg/mL)	Range (pg/mL)
Mouse serum (n=16)	2,690.2	2,066.4-3,200.2

Sensitivity

The minimum detectable dose of mouse Osteoprotegerin/TNFRSF11B is 0.04 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, mouse serum samples containing concentration of mouse Osteoprotegerin/TNFRSF11B were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. Cell culture supernatants samples were spiked with high concentrations of mouse Osteoprotegerin/TNFRSF11B in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

		Mouse serum (PT 5)	Cell culture supernatants (PT 1-ef)
1:2	Average% of Expected	100	106
	Range (%)	-	87-126
1:4	Average% of Expected	96	105
	Range (%)	90-103	89-123
1:8	Average% of Expected	100	104
	Range (%)	86-108	91-112
1:16	Average% of Expected	104	109
	Range (%)	96-114	89-120

References

1. Simonet WS, et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell*. 1997;89(2):309-319.
2. Emery JG, et al. Osteoprotegerin is a receptor for the cytotoxic ligand TRAIL. *J Biol Chem*. 1998;273(23):14363-14367.
3. Yasuda H, et al. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proc Natl Acad Sci U S A*. 1998;95(7):3597-3602.