

colorimetric sandwich ELISA kit datasheet

For the quantitative detection of mouse TIMP1 in serum, plasma and cell culture supernatants.

general information

Catalogue Number	KE10039
Product Name	TIMP1 ELISA Kit
Species cross-reactivity	Mouse TIMP1
Range (calibration Range)	31.25– 2000 pg/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	21857 (Mouse)
SwissProt	P12032 (Mouse)

kit components & storage

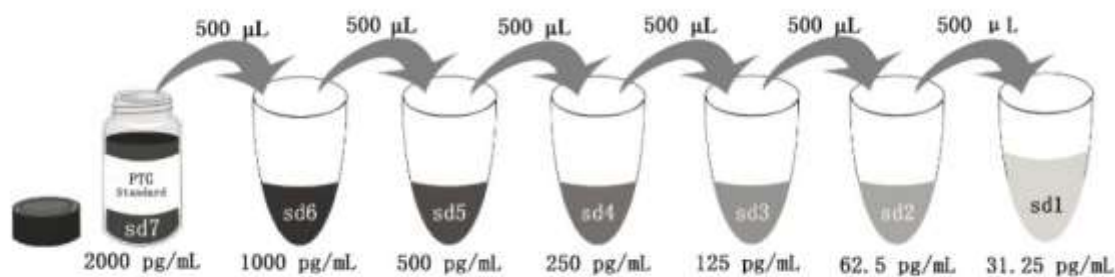
Microplate - antibody coated 96-well Microplate (8 well × 12 strips)	1 plate	Store at 2-8°C for six months
Standard – 2000 pg/bottle; lyophilized*	2 bottles	Store at 2-8°C for six months
Detection antibody, biotinylated (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Streptavidin-HRP (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 3-eg - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Detection Diluent - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Stop Solution - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Plate Cover Seals	3 pieces	

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

Sample Diluent PT 3-eg is for standard and samples.

Detection Diluent is for Detection antibody and Streptavidin-HRP antibody.

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

product description

KE10039 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The TIMP1 ELISA kit is to be used to detect and quantify protein levels of endogenous TIMP1. The assay recognizes mouse TIMP1. An antibody specific for TIMP1 has been pre-coated onto the microwells. The TIMP1 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for mouse TIMP1 is added to detect the captured mouse TIMP1 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 450nm with the correction wavelength set at 630 nm.

background

TIMP1 is a member of the family of matrix metalloproteinase inhibitors, which contains four members (TIMP1, TIMP2, TIMP3, and TIMP4). Tissue inhibitors of metalloproteinases (TIMPs) are multifaceted molecules that exhibit properties beyond their classical proteinase inhibitory function. TIMP1 has several MMP-independent functions such as modulation of angiogenesis, promotion of cell proliferation, and inhibition of apoptosis. TIMP1 plays important role in cell cycle regulation and cancer progression. Recently, clinical studies have shown that the aberrant expression of TIMP1 is associated with an unfavorable prognosis in a series of tumors, such as gastric cancer, papillary thyroid carcinoma, cutaneous melanoma and breast cancer. In pregnancy, TIMP1 plays a regulatory role in the process of implantation, particularly the cytotrophoblast invasion of the uterine endometrium. In pregnancy, TIMP1 plays a regulatory role in the process of implantation, particularly the cytotrophoblast invasion of the uterine endometrium.

sample preparation

The serum, plasma and cell culture supernatants may require proper dilution to fall within the range of the assay. The serum, plasma are suggested diluted 1:4 or 1:8. The cell culture supernatants are suggested diluted 1:4 or 1:8.

safety notes

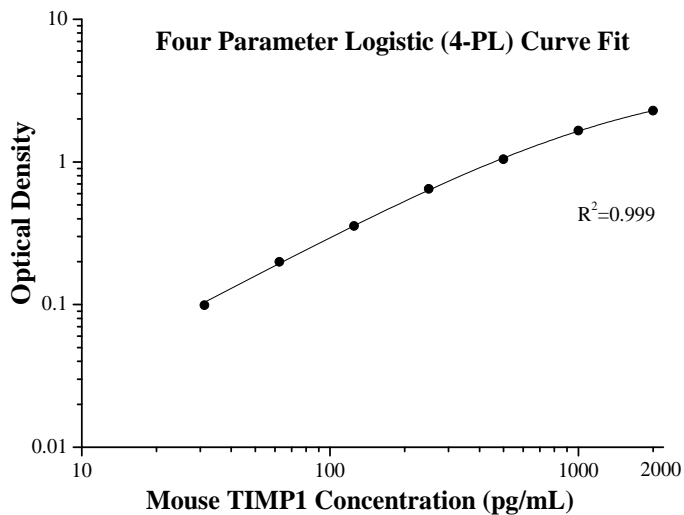
This product is sold for lab research and development use ONLY and not for use in humans or animals.
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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.				

typical 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	0.104	0.104	—
	0.103		
31.25	0.204	0.203	0.099
	0.203		
62.5	0.301	0.303	0.199
	0.304		
125	0.464	0.459	0.356
	0.454		
250	0.749	0.751	0.648
	0.753		
500	1.151	1.148	1.045
	1.145		
1000	1.794	1.761	1.657
	1.727		
2000	2.391	2.390	2.286
	2.388		

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.

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (pg/mL)	1,041.5	251.9	64.8	1,031.1	274.4	74.4
SD	40.3	6.8	1.9	56.8	13.6	4.2
CV%	3.8	2.7	2.9	5.5	4.9	5.6

recovery

The recovery of TIMP1 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	1:60	102	76-113
	1:120	85	77-106
Cell culture supernatants	1:60	97	79-107
	1:120	90	81-102

sample value

Sample Type	Mean of Detectable (pg/mL)	Range (pg/mL)
Mouse serum (n=16)	1,225	572-2,370

Cell culture supernatants:

L-929 mouse fibroblast cells (1×10^6 cells/mL) were cultured for 3 days in 50% DMEM and 50% F12 supplemented with 10% fetal bovine serum, 2.5 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot of the cell culture supernate was removed, assayed for mouse TIMP-1, and measured 7,285 pg/mL.

sensitivity

The minimum detectable dose of mouse TIMP1 is 1.2 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 mouse serum samples were initially diluted 1:2. The cell culture supernatants samples were initially diluted 1:2.)

		Mouse serum	Cell culture supernatants
1:2	Average% of Expected	100	100
	Range (%)	-	-
1:4	Average% of Expected	106	110
	Range (%)	102-109	108-111
1:8	Average% of Expected	110	109
	Range (%)	103-117	102-114
1:16	Average% of Expected	103	112
	Range (%)	80-118	105-119

references

1. Stetler-Stevenson WG. et al.(2008) Sci Signal.1(27):re6.
2. Batra J. et al.(2012) 287(19):15935-46.
3. Kim YS. et al. (2012) BMB Rep. 45(11):623-8.
4. Graham CH. et al. (1991) J Cell Physiol. 148(2):228-34.
5. Song G. et al. (2016) J Exp Clin Cancer Res. 35(1):148.