

## Human TIMP1 Sandwich ELISA Kit Datasheet

For the quantitative detection of human TIMP1 concentrations in serum, plasma, cell culture supernatants and saliva.

### General Information

Catalogue Number	KE00166
Product Name	Human TIMP1 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	62.5-4000 pg/mL
Tested applications	Quantification ELISA

### Database Links

Entrez Gene	7076
SwissProt	P01033

### Kit Components & Storage

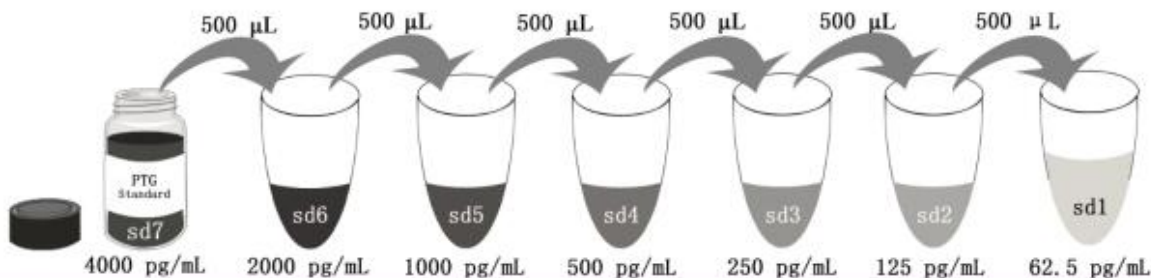
Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	<b>Unopened Kit:</b> Store at 2-8°C for 6 months or -20°C for 12 months.  <b>Opened Kit:</b> All reagents stored at 2-8°C for 7 days.  <b>Please use a new standard for each assay.</b>
Protein standard - 4000 pg/bottle; lyophilized*	2 bottles	
Detection antibody, biotinylated (100X) - 120 µL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 1 - 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 PT1 is for protein standard and samples.

Detection Diluent is for Detection antibody and Streptavidin-HRP.

\*Add 1mL Sample Diluent PT 1 in protein standard. This reconstitution gives a stock solution of 4000 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 1	1000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

## Product Description

KE00166 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 human 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 human TIMP1 is added to detect the captured human 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 450 nm 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

Different samples may require proper dilution to fall within the range of the assay. 1:300 or 1:600 dilution is recommended for

serum or plasma, 1:20 or 1:40 dilution is recommended for cell culture supernatants, 1:100 or 1:200 dilution is recommended for saliva.

## Safety Notes

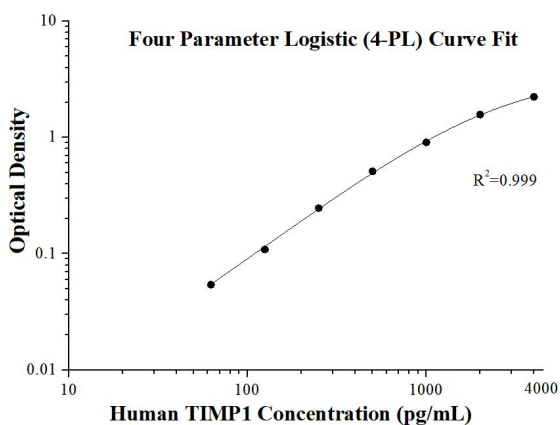
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

## 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	0.102 0.104	0.103	-
62.5	0.158 0.156	0.157	0.054
125	0.22 0.203	0.212	0.109
250	0.331 0.368	0.350	0.247
500	0.614 0.614	0.614	0.511
1000	0.971 1.047	1.009	0.906
2000	1.705 1.65	1.678	1.575
4000	2.372 2.309	2.341	2.238

## 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	1,313.4	54.2	4.1
2	20	815.8	50.8	6.2
3	20	423.2	20.6	4.9

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	1,181.1	99.7	8.4
2	24	851.8	63.6	7.5
3	24	486.7	39.3	8.1

## 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 (%)
Human serum	1:300	92	75-111
	1:600	95	79-108
Cell culture supernatants	1:40	95	75-110
	1:80	89	83-98
Saliva	1:400	87	76-101
	1:800	97	79-106

## Sample Values

Serum and saliva samples from healthy volunteers were evaluated for TIMP1 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (ng/mL)	Range (ng/mL)
Human serum (n=16)	335.2	252.6-556.3
Saliva (n=9)	84.8	12.0-284.8

Human peripheral blood mononuclear cells ( $1 \times 10^6$  cells/mL) were cultured in RPMI supplemented with 8% fetal bovine serum,  $50 \mu\text{M}$   $\beta$ -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 supernatants were removed on days 5 assayed for levels of human TIMP1.

Condition	Day 5 (ng/mL)
Unstimulated	54.7
Stimulated	102.6

## Sensitivity

The minimum detectable dose of human TIMP1 is 6.9 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, human serum, cell culture supernatants and saliva 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 samples were initially diluted 1:10.

The saliva samples were initially diluted 1:50)

		Human serum	Cell culture supernatants	Saliva
1:2	Average% of Expected	100	100	100
	Range (%)	-	-	-
1:4	Average% of Expected	105	100	103
	Range (%)	102-107	96-104	99-107
1:8	Average% of Expected	105	95	108
	Range (%)	88-116	92-100	106-109
1:16	Average% of Expected	102	89	98
	Range (%)	94-110	82-97	94-105

## 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.