

## Speedy™ Human/Cynomolgus Monkey TIMP1 One-Step ELISA Kit Datasheet

Please read it entirely before use

**Catalogue Number:** SE50010

**Size:** 96T

**Sensitivity:** 2.4 pg/mL

**Range:** 15.6-1000 pg/mL

**Usage:** For the quantitative detection of human/cynomolgus monkey TIMP1 concentrations in serum, plasma, cell culture supernatant and saliva.

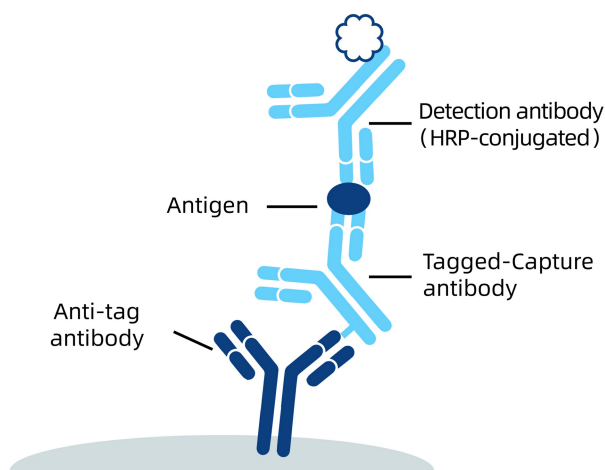
**This product is for research use only and not for use in human or animal therapeutic or diagnostic.**

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## 1. Background

Metalloproteinase inhibitor 1 (TIMP1) is also named as Erythroid-potentiating activity (EPA), Fibroblast collagenase inhibitor (Collagenase inhibitor) and CLGI. TIMP1 is one of the tissue inhibitor members of the metalloproteinase family. TIMP1 regulates the balance of matrix remodeling during the degradation of extracellular matrix by inhibiting the proteolytic activity of matrix metalloproteinases (MMPs). TIMP1 promotes the occurrence and development of GC, which is consistent with previous reports about TIMP1 as an oncogene in other cancers, including breast cancer, colon cancer, glioblastoma, and non-small cell lung cancer. TIMP1 is reportedly as a tumor-secreted protein that could be detected in the serum. In a diabetic environment, TIMP1 initially binds to TFRC, leading to increased TFRC expression, thereby heightening osteoblast sensitivity to ferroptosis.

## 2. Principle



An anti-tag antibody is pre-coated onto the bottom of wells. After adding antigen or samples, Tagged-Capture antibody and HRP-conjugated detection antibody, a sandwich complex is formed in the solution. TMB acts as a HRP substrate, and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns the solution yellow. The color intensity is proportional to the quantity of bound protein, which is measurable at 450 nm with the correction wavelength set at 630 nm.

## 3. Required Materials

- 3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.
- 3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.
- 3.3 Plate washer: automated or manual.
- 3.4 Absorbent paper towels.
- 3.5 Glass or plastic tubes to prepare standard and sample dilutions.
- 3.6 Beakers and graduated cylinders.
- 3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended. Proteintech data analysis website, <https://www.ptgcn.com/products/elisa-kits/>.
- 3.8 Microplate thermostatic shaker.

## 4. Kit Components and Storage

<b>Microplate</b> - 96 well microplate precoated an anti-tag antibody (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>
<b>Protein standard</b> - 2000 pg/bottle; lyophilized	2 bottles	
<b>Capture antibody (100×)</b> - 60 uL/vial*	1 vial	
<b>Detection antibody, HRP-Conjugated (100×)</b> - 60 uL/vial*	1 vial	
<b>Sample Diluent PT 4B1</b> - 30 mL/bottle	1 bottle	
<b>Detection Diluent</b> - 15 mL/bottle	1 bottle	
<b>Wash Buffer Concentrate (20×)</b> - 30 mL/bottle	1 bottle	
<b>Tetramethylbenzidine Substrate (TMB)</b> - 12 mL/bottle	1 bottle	
<b>Stop Solution</b> - 12 mL/bottle	1 bottle	
<b>Plate Cover Seals</b>	4 pieces	

\* Centrifugation immediately before use

## 5. Safety Notes

- 5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.
- 5.2 Do not use the kit after the expiration date.
- 5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.
- 5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.
- 5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

## 6. Sample Collection and Storage

- 6.1 Serum: Allow blood samples to clot for 30 minutes, followed by centrifugation for 15 minutes at 1000×g. Clear serum can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.
- 6.2 Plasma: Use EDTA, heparin, or citrate as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.
- 6.3 Cell Culture Supernatant: Remove particulates by centrifugation for 5 minutes at 500×g and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
- 6.4 Saliva: Collect saliva samples and centrifuge for 5 minutes at 10,000×g. Collect the aqueous layer, assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

## 7. Regent Preparation

**7.1 Wash Buffer (1X):** If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

**7.2 Antibody Cocktail (1X):** Dilute 100X capture antibody and 100X HRP-conjugated detection antibody using Detection Diluent prior to assay. Suggested 1:100 dilution: 50  $\mu$ L 100X capture antibody + 50  $\mu$ L 100X Detection Antibody, HRP-conjugated + 4,900  $\mu$ L Detection Diluent. Mix gently but thoroughly.

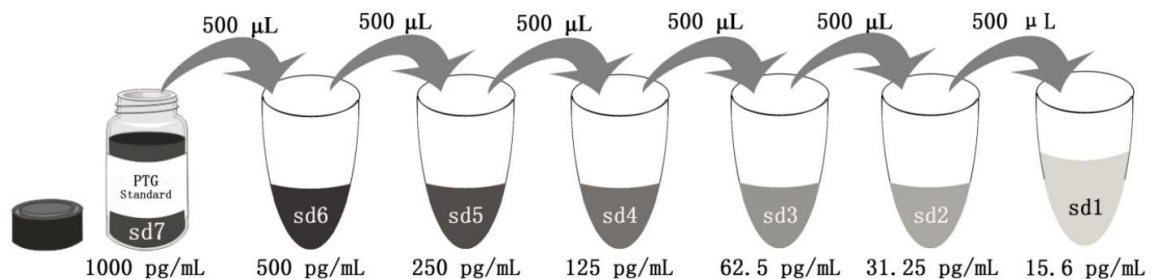
**7.3 Sample Dilution:** Different samples should be diluted with corresponding Sample Diluent. The minimum required dilution factor was 1:2; consequently, no more than 25  $\mu$ L of undiluted sample was added per well.

Samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:200 or 1:400 is recommended for human serum and plasma; 1:1,600 or 1:3,200 is recommended for cynomolgus monkey serum and plasma; 1:40 or 1:80 is recommended for cell culture supernatant; 1:400 or 1:800 is recommended for saliva.

### 7.4 Standard Serial Dilution:

Add 2 mL Sample Diluent PT 4B1 in protein standard.



Add # $\mu$ L of Standard diluted in the previous step	—	500 $\mu$ L	500 $\mu$ L	500 $\mu$ L	500 $\mu$ L	500 $\mu$ L	500 $\mu$ L
# $\mu$ L of Sample Diluent PT 4B1	<b>2000 <math>\mu</math>L</b>	500 $\mu$ L	500 $\mu$ L	500 $\mu$ L	500 $\mu$ L	500 $\mu$ L	500 $\mu$ L
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

## 8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody, HRP-conjugated antibody can be used immediately). To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

8.1 Preset the layout of the microplate, including control group, standard group and sample group, take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Add 50 µL standard or sample to appropriate wells. To avoid high background always add samples or standards to the well before the addition of antibody cocktail.

8.3 Add 50 µL 1× Antibody Cocktail solution (refer to Reagent Preparation 7.2) to each well. Seal plate with cover seal and incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 1 hour (incubate at 37°C for 2 hours is recommended if thermostatic shaker is not available).

### 8.4 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1× Wash Buffer, using at least 350-400 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.5 Add 100 µL TMB substrate solution to each well, protected from light. Incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 15 to 20 minutes. (Substrate Solution should remain colorless until added to the plate.)

8.6 Add 100 µL Stop Solution to each well in the same order as addition of the TMB substrate. Note: Avoid skin and eye contact with the Stop solution.

8.7 Read results immediately on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.8 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, four-parameter logistic curve-fit (4-PL) analysis is recommended. If the samples have been diluted, the fitting result must be multiplied by the dilution factor used.

## Procedure summary

Add 50 µL standard or sample to appropriate wells



Add 50 µL antibody cocktail solution (1x) to each well



Incubate at 37 °C on a microplate thermostatic shaker set at 400 rpm for 1 hour



Aspirate and wash each well four times with 400µL 1 x Wash Buffer



Add 100 µL TMB substrate solution to each well



Incubate at 37 °C on a microplate thermostatic shaker set at 400 rpm for 15minutes

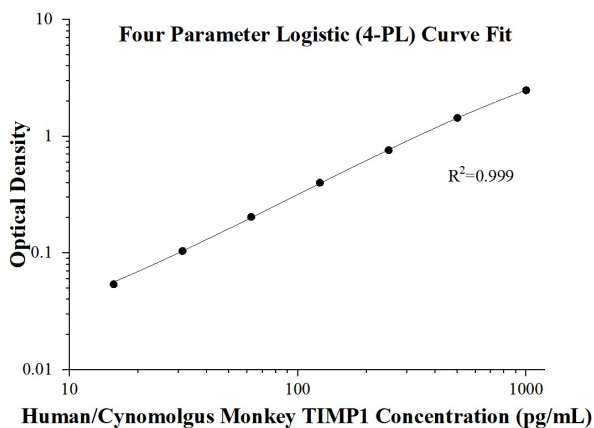


Add 100 µL Stop Solution to each well and Read OD immediately

## 9. Validation Data

### 9.1 Standard curve

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	0.0358 0.0361	0.03595	-
15.6	0.0884 0.0914	0.0899	0.05395
31.25	0.1399 0.1397	0.1398	0.10385
62.5	0.2425 0.2362	0.23935	0.2034
125	0.4345 0.4373	0.4359	0.39995
250	0.8094 0.7835	0.79645	0.7605
500	1.4836 1.4668	1.4752	1.43925
1000	2.5365 2.5029	2.5197	2.48375

## 9.2 Precision

**Intra-assay Precision** (Precision within an assay) Three samples of known concentration were tested 8 times on one plate to assess intra-assay precision.

**Inter-assay Precision** (Precision between assays) Three samples of known concentration were tested in 16 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	8	443.5	10.6	2.4	1	16	447.7	14.7	3.3
2	8	115.2	1.9	1.7	2	16	116.2	2.3	2.0
3	8	35.4	0.4	1.2	3	16	36.2	1.6	4.3

## 9.3 Recovery

The recovery of human/cynomolgus monkey TIMP1 spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Human plasma	1:800	105	101-114
	1:1,600	105	98-110
Cynomolgus monkey serum	1:3,200	93	90-96
	1:6,400	101	98-104
Cell culture supernatant	1:160	92	86-97
	1:320	96	94-98
Saliva	1:1,600	103	98-111
	1:3,200	102	99-106

## 9.4 Sample values

**Plasma/Serum/Saliva** - human plasma, cynomolgus monkey serum and human saliva samples were evaluated for the presence of TIMP1 in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)
Human plasma (n=16)	137.7	79.5-235.7
Cynomolgus monkey serum (n=8)	670.1	554.5-811.1
Human saliva (n=8)	136.7	91.8-191.7

**Cell culture supernatant** - human peripheral blood mononuclear cells ( $1 \times 10^6$  cells/mL) were cultured in DMEM supplemented 10% fetal bovine serum, 5  $\mu$ M  $\beta$ -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin and 100  $\mu$ g/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10  $\mu$ g/mL PHA for 1 day. Aliquots of the cell culture supernates were removed and assayed for levels of TIMP1.

Condition	Day 1 (pg/mL)
Unstimulated	10,904.4
Stimulated	23,560.6

## 9.5 Sensitivity

The minimum detectable dose of human/cynomolgus monkey TIMP1 is 2.4 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

## 9.6 Linearity

To assess the linearity of the assay, cell culture supernatant samples were spiked with high concentrations of human TIMP1 and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. Human plasma, cynomolgus monkey serum and saliva samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

(The human plasma was initially diluted 1:100. The cynomolgus monkey serum was initially diluted 1:800. The cell culture supernatant was initially diluted 1:20. The saliva was initially diluted 1:200.)

		Human plasma	Cynomolgus monkey serum	Cell culture supernatant	Saliva
1:2	Average% of Expected	100	100	99	100
	Range (%)	-	-	98-100	-
1:4	Average% of Expected	101	99	100	104
	Range (%)	100-102	97-101	99-100	103-105
1:8	Average% of Expected	105	104	106	108
	Range (%)	104-106	102-106	104-109	105-110
1:16	Average% of Expected	98	99	107	103
	Range (%)	98-99	98-100	106-108	95-110

## 9.7 Specificity

This assay recognizes natural and recombinant human/cynomolgus monkey TIMP1.

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant human:

MMP-2

MMP-3

TIMP2

A sample containing 50 ng/mL of the recombinant mouse TIMP1 reads as 35.3 pg/mL (0.07% cross-reactivity).

## 10. References

1. Song, Guohe et al. Journal of experimental & clinical cancer research : CR vol. 35,1 148.
2. Akama, Takeshi, and Tae-Hwa Chun. The Journal of biological chemistry vol. 293,17 (2018): 6603-6610.
3. Dantas, Ezequiel et al. Clinical and translational medicine vol. 13,10 (2023): e1391.
4. Peng, Bo et al. Molecular medicine (Cambridge, Mass.) vol. 30,1 226.