OriGene

Human TIMP-1 Immunoassay

Catalog Number: EA800105

For the quantitative determination of human TIMP-1 concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

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BACKGROUND

Matrix Metalloproteinases (MMPs) are zinc-dependent endopeptidases that catalyze degradation of extracellular matrix proteins, thereby controlling such processes as development, tissue remodeling, wound healing and tumor metastasis . The activity of MMPs is controlled by regulation of expression and secretion, by proteolytic activation of pro-enzymes and by the Tissue Inhibitors of Metalloproteinases (TIMPs). TIMP-1 is a 184 amino acid residue glycosylated protein, though glycosylation is not necessary for activity . It has 12 cysteines (conserved among all TIMPs) that form disulfide bonds in a pattern that gives distinct N- and C-terminal domains . The N-terminal domain contains sites that bind to the MMP substrate-binding site . Binding of TIMP-1 does not leave a peptide bond in position for proteolysis and is not cleaved . The TIMP/MMP complex can dissociate to yield enzyme and active TIMP-1 . The C-terminal domain binds to an external site on MMPs, increasing overall affinity. TIMP-1 binds with high affinity to the inactive pro-MMP-9, forming a complex in which TIMP-1 retains its ability to inhibit the activity of another active MMP via its N-terminal domain

TIMP-1 is widely synthesized by many cells and tissues . Transcription of the TIMP-1 gene is induced by pro-inflammatory cytokines (IL-1, IL-6, OSM, LIF and TNF-α), TGF-β1 and phorbol esters . Many physiological functions of TIMP-1 are closely tied to the functions of MMPs, and an improper balance of MMP and TIMP production correlates with pathological conditions such as arthritis, tumor growth and metastasis . On the other hand, TIMP-1 was independently discovered as an erythroid potentiating activity , an activity that appears to be functionally distinct from MMP inhibitory activity . TIMP-1 binds to certain cell lines and is translocated to the nucleus . It inhibits apoptosis in B cells , further suggesting that it independently functions in multiple ways to support survival and growth of cells in contrast to its function of inhibition of MMPs.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for TIMP-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TIMP-1 present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for TIMP-1 is added to detect the captured TIMP-1 protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. 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.

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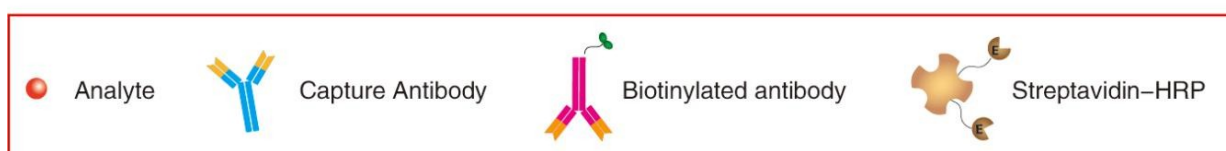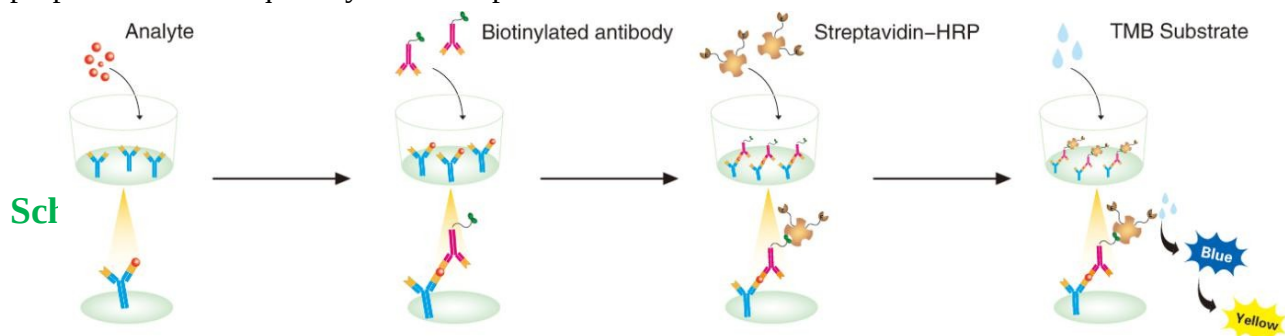
TECHNICAL HINTS AND LIMITATIONS

1. This ELISA should not be used beyond the expiration data on the kit label.
2. To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3. To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4. A thorough and consistent wash technique is essential for proper assay performance.
5. A standard curve should be generated for each set of samples assayed.
6. It is recommended that all standards and samples be assayed in duplicate.
7. Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8. In order to ensure the accuracy of the results, the standard curve should be made every time.

PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL

Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2 – 8℃**
Standard-lyophilized,2000 pg/vial upon reconstitution	2 vials	Aliquot and Store at -20°C** for six months
Concentrated Biotin-Conjugated antibody(100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Concentrated Streptavidin-HRP solution(100X) - 120 ul/vial	1 vial	Store at 2-8°C** for six months
Standard /Sample Diluent - 16 ml/vial	1 bottle	Store at 2-8°C** for six months
Biotin-Conjugate antibody Diluent - 16 ml/vial	1 bottle	Store at 2-8°C** for six months
Streptavidin-HRP Diluent - 16 ml/vial	1 bottle	Store at 2-8°C** for six months
Wash Buffer Concentrate (20x) - 30 ml/vial	1 bottle	Store at 2-8°C** for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C** for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C** for six months
Plate Cover Seals	4 pieces	

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Pipettes and pipette tips.

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3. Deionized or distilled water.
4. Squirt bottle, manifold dispenser, or automated microplate washer.
5. 500 mL graduated cylinder.

SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000×g to remove debris. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
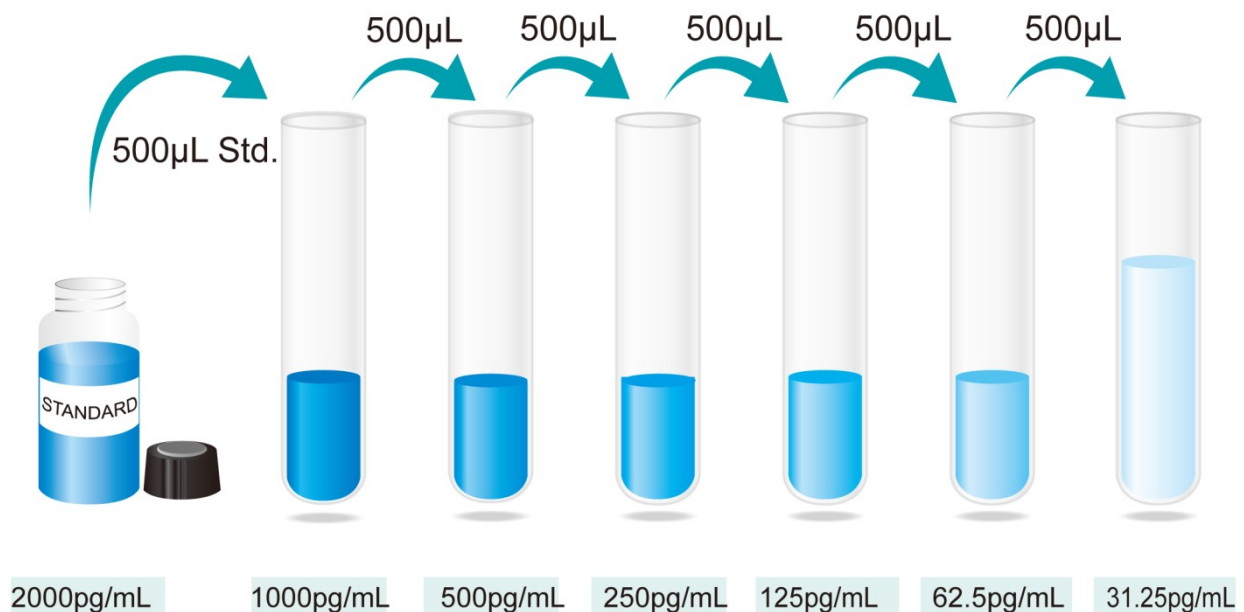**Serum** - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature

or overnight at 2-8℃. Centrifuge approximately for 15 minutes at 1000×g. Assay immediately or

aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Note: The normal human serum or plasma samples are suggested to make a 1:2 dilution.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25℃) before use.

2. **Wash Buffer** - Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

3. **Standard/Sample** - Reconstitute the Standard with 1.0mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 2000pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 500μL of Standard/Sample Diluent into 1000pg/ml tube and the remaining tubes. Use the stock solution of 2000pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 2000 pg/mL standard serves as the high standard. The Standard/Sample Diluent serves as the zero standard (0 pg/mL).

500μL	500μL	500μL	500μL	500μL

500μL Std.

STANDARD

2000pg/mL	1000pg/mL	500pg/mL	250pg/mL	125pg/mL	62.5pg/mL	31.25pg/mL

Preparation of TIMP-1 standard dilutions

***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**

4. **Working solution of Biotin-Conjugate anti-human TIMP-1 antibody:** Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

   ***The working solution should be used within one day after dilution.**

5. **Working solution of Streptavidin-HRP**: Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.

   *The working solution should be used within one day after dilution.

ASSAY PROCEDURE

Prepare all reagents and standards as directed. Wash the plate 3 times before assay.

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Add 100μl standard or samples to each well, incubate 90 minutes,37℃.

⇩ Aspirate and wash 4 times

Add 100μl working solution of Biotin-Conjugate anti-human TIMP-1 antibody to each well, incubate 60 minutes,37℃.

⇩ Aspirate and wash 4 times

Add 100μl working solution of Streptavidin-HRP to each well, incubate 30 minutes,37℃.

⇩ Aspirate and wash 5 times

| Add 100μl Substrate solution to each well, incubate 15 minutes,37℃.Protect from light. |

⇩

| Add 50μl Stop solution to each well. Read at 450nm within 30 minutes. |

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.

2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.

3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.

4. The data may be linearized by plotting the log of the TIMP-1 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

5. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the TIMP-1 ELISA

Standard(pg/ml)	OD.	OD.	Average	Corrected
0	0.079	0.078	0.079	---
31.25	0.096	0.102	0.099	0.021
62.5	0.106	0.107	0.107	0.028
125	0.161	0.163	0.162	0.084
250	0.280	0.282	0.281	0.203

500	0.651	0.650	0.651	0.572
1000	1.325	1.326	1.326	1.247
2000	2.788	2.786	2.787	2.709
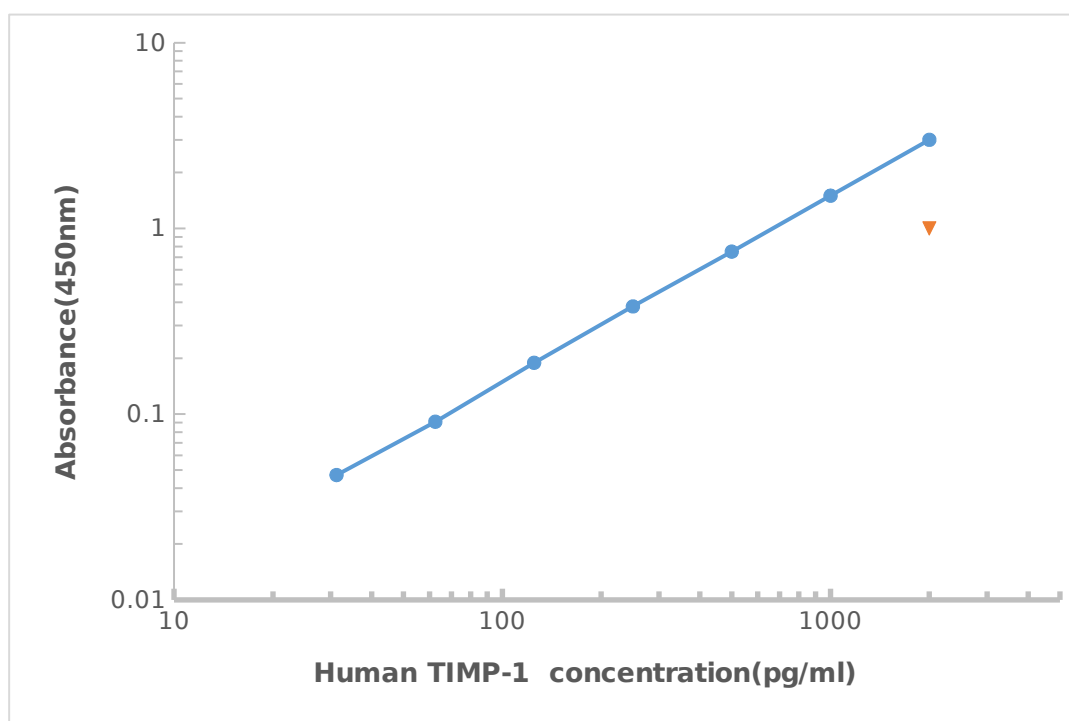
Representative standard curve for TIMP-1 ELISA.

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 15pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant human TIMP-1. The factors listed below were prepared at 100ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Factors assayed for cross-reactivity

Recombinant human	Recombinant mouse	Recombinant porcine
MMP-1		
MMP-2		
MMP-3		
TIMP-2		
TIMP-3		
TIMP-4		

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

RECOVERY： The recovery of TIMP-1 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of TIMP-1 in two matrices

Sample Type	Average % of Expected Range (%)	Range (%)
Citrate plasma	92	84-101
Cell culture supernatants	96	89-105

LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of TIMP-1 in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

Dilution ratio	Recovery (%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	96	102
	Range (%)	88-102	94-111

1:4	Average% of Expected	97	105
	Range (%)	90-106	97-113
1:8	Average% of Expected	95	103
	Range (%)	86-104	95-114
1:16	Average% of Expected	96	106
	Range (%)	85-105	98-115

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