Quantikine® ELISA

Mouse TIMP-1 Immunoassay

Catalog Number MTM100

For the quantitative determination of mouse Tissue Inhibitor of Metalloproteinases 1 (TIMP-1) concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

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INTRODUCTION

Matrix metalloproteinases (MMPs), also called matrixins, constitute a family of zinc and calcium dependent endopeptidases that function in the breakdown of extracellular matrix and in the processing of a variety of biological molecules. They play an important role in many normal physiological processes such as embryonic development, morphogenesis, reproduction and tissue remodeling (1). They also participate in many pathological processes such as arthritis, cancer and cardiovascular disease (2). While the amounts of newly synthesized MMPs are regulated mainly at the levels of transcription, the proteolytic activities of existing MMPs are controlled through both the activation of proenzymes or zymogens and the inhibition of active enzymes by endogenous inhibitors such as α_2-macroglobulins and tissue inhibitors of metalloproteinases (TIMPs).

The mammalian TIMP family includes four members that share structural similarity (3). All TIMP proteins have 12 conserved cysteine residues that form six intrachain disulfide bonds, resulting in an extremely stable protein with six loops. The TIMP protein has two structurally and functionally distinct domains: the N-terminal domain consisting of loops 1-3 that are responsible for tight but non-covalent binding to the active MMPs in a 1:1 stoichiometry; and the C-terminal domain consisting of loops 4-6 that enhances the enzyme-inhibitor interactions. In the case of TIMP-1, the C-terminal domain has also been shown to bind the hemopexin-like domain of pro MMP-9. TIMP-1 stimulates erythropoiesis, inhibits angiogenesis and is an anti-apoptotic agent for B cells. These TIMP-1 functions may be independent of MMP inhibition (4-6).

Mouse TIMP-1 is a 28-35 kDa secreted glycoprotein (6, 7). The protein is synthesized as a 205 amino acid (aa) precursor that contains a 24 aa signal peptide and a 181 aa mature form (8, 9). Mouse TIMP-1 shares 85%, 77%, 68%, and 67% aa sequence identity with rat, human, porcine, and canine TIMP-1, respectively (10-13). Among the three known mouse TIMPs, TIMP-1 shares 39% and 38% aa sequence identity with TIMP-2 and TIMP-3, respectively (14, 15). TIMP-1 is widely expressed in many cells including fibroblasts, osteoblasts, endothelial cells, granulosa cells, dendritic cells, vascular smooth muscle cells, adipocytes, and monocytes (6, 16-19).

The Quantikine Mouse TIMP-1 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse TIMP-1 in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant mouse TIMP-1 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural mouse TIMP-1 showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative levels of natural mouse TIMP-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse TIMP-1 has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any mouse TIMP-1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse TIMP-1 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of mouse TIMP-1 bound in the initial step. The sample values are then read off the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- 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.
- For best results, pipette reagents and samples into the center of each well.
- It is recommended that the samples be pipetted within 15 minutes.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse TIMP-1 Microplate	891102	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse TIMP-1.	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 °C.*
Mouse TIMP-1 Standard	891104	Recombinant mouse TIMP-1 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store for up to 1 month at 2-8 °C.*
Mouse TIMP-1 Control	891105	Recombinant mouse TIMP-1 in a buffered protein base with preservatives; lyophilized. The assay value of the Control should be within the range specified on the label.	
Mouse TIMP-1 Conjugate	891103	12 mL of a polyclonal antibody against mouse TIMP-1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-21	895215	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-17	895512	21 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric acid.	
Plate Sealers	N/A	4 adhesive strips.	

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

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- **Polypropylene** test tubes for dilution of standards and samples.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Heparin and citrate plasma have not been validated for use in this assay.*
Grossly hemolyzed or lipemic samples may not be suitable for use in this assay.

SAMPLE PREPARATION

Use polypropylene tubes.

Cell culture supernate sample may require dilution.

Serum and plasma samples require a least a 3-fold dilution prior to assay. A suggested 3-fold dilution is 40 µL of sample + 80 µL of Calibrator Diluent RD5-17.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

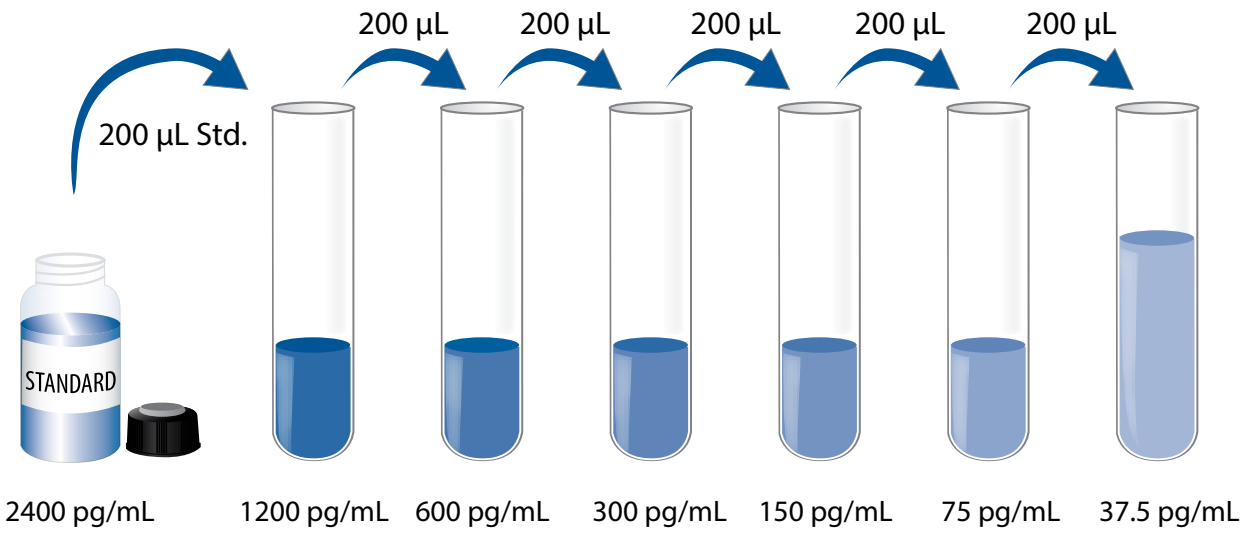
Mouse TIMP-1 Control - Reconstitute the Control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the Control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Mouse TIMP-1 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse TIMP-1 Standard with Calibrator Diluent RD5-17. Do not substitute other diluents. This reconstitution produces a stock solution of 2400 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD5-17 into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted mouse TIMP-1 Standard serves as the high standard (2400 pg/mL). Calibrator Diluent RD5-17 serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, Control, and samples be assayed in duplicate.

1. Prepare all reagents, standard dilutions, control, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1-21 to each well.
4. Add 50 µL of Standard, Control, or sample* per well. Mix by gently tapping the plate frame for 1 minute. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Mouse TIMP-1 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

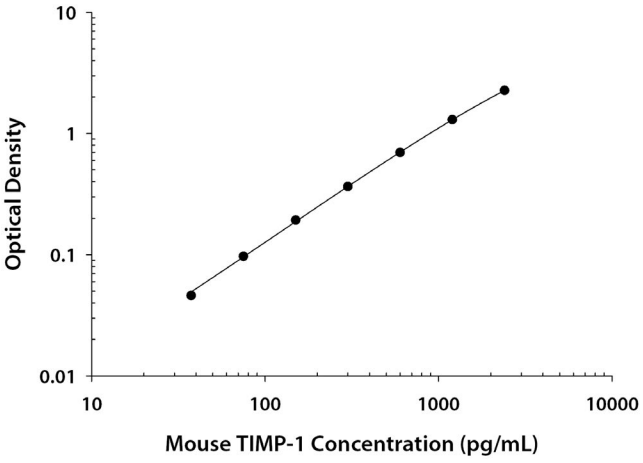
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create 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. The data may be linearized by plotting the log of the mouse 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.

TYPICAL DATA

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



(pg/mL)	O.D.	Average	Corrected
0	0.031 0.031	0.031	—
37.5	0.077 0.077	0.077	0.046
75	0.126 0.131	0.128	0.097
150	0.224 0.224	0.224	0.193
300	0.393 0.399	0.396	0.365
600	0.728 0.729	0.728	0.697
1200	1.294 1.369	1.332	1.301
2400	2.245 2.342	2.294	2.263

PRECISION

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

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three techniciansusing two lots of kit components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	91.8	282	721	89.7	293	721
Standard deviation	9.4	12.6	23.0	6.5	22.1	41.4
CV (%)	10.2	4.5	3.2	7.2	7.5	5.7

RECOVERY

The recovery of mouse TIMP-1 spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=6)	102	92-112%
Serum* (n=6)	101	84-117%
EDTA plasma* (n=4)	107	92-119%

*Samples were diluted prior to assay.

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of mouse TIMP-1 in each matrix were diluted with Calibrator Diluent and assayed.

		Cell culture supernates (n=5)	Serum* (n=6)	EDTA plasma* (n=4)
1:2	Average % of Expected	96	97	99
	Range (%)	91-102	93-107	97-103
1:4	Average % of Expected	98	98	104
	Range (%)	90-103	93-109	95-107
1:8	Average % of Expected	97	100	107
	Range (%)	85-103	92-113	103-117
1:16	Average % of Expected	98	102	109
	Range (%)	89-108	94-113	101-116

*Samples were diluted prior to assay.

SENSITIVITY

Seven assays were evaluated and the minimum detectable dose (MDD) of mouse TIMP-1 ranged from 1.4-3.5 pg/mL. The mean MDD was 2.1 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant mouse TIMP-1 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse TIMP-1 in this assay.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=20)	2350	1280-3650	730
EDTA plasma (n=20)	2270	1250-3390	560

Cell Culture Supernates:
L-929 mouse fibroblast cells (1 x 10^6 cells/mL) were cultured for 3 days in MEM supplemented with 10% equine serum and stimulated with 2.5 ng/mL LPS. An aliquot of the cell culture supernate was removed, assayed for mouse TIMP-1, and measured 26.6 ng/mL.

Mouse lung conditioned media (1-2 mm pieces in 40 mL of medium) were cultured for 7 days in RPMI supplemented with 10% fetal calf serum. An aliquot of the cell culture supernate was removed, assayed for mouse TIMP-1, and measured 121 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse TIMP-1.

The factors listed below were prepared at 50 ng/mL or 500 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a recombinant mouse TIMP-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:	Recombinant human:	
MMP-3	MMP-1	MMP-12
MMP-9 (pro)	MMP-2	MMP-13
MMP-9 (active)	MMP-3	TIMP-1
	MMP-7	TIMP-2
	MMP-8	TIMP-3
	MMP-9	TIMP-4
	MMP-10	

Recombinant rat TIMP-1 cross-reacts approximately 0.04% in this assay.

This assay detects approximately 80% of the recombinant mouse TIMP-1 complexed with the pro-form of recombinant mouse MMP-9 and approximately 60% when complexed with active recombinant mouse MMP-9.

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