Magnetic Luminex® Performance Assay

Human TIMP Multiplex Kit

Catalog Number LKTM003

For the simultaneous quantitative determination of the concentrations of multiple human Tissue Inhibitors of Metalloproteinases (TIMPs) in cell culture supernates, serum, plasma, saliva, urine, and human milk.

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

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Manufactured and Distributed by:

USA R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413
TEL: 800 343 7475 612 379 2956
FAX: 612 656 4400
E-MAIL: info@bio-techne.com

Distributed by:

Europe | Middle East | Africa Bio-Techne Ltd.
19 Barton Lane, Abingdon Science Park
Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449
FAX: +44 (0)1235 533420
E-MAIL: info.emea@bio-techne.com

China Bio-Techne China Co., Ltd.
Unit 1901, Tower 3, Raffles City Changning Office,
1193 Changning Road, Shanghai PRC 200051
TEL: +86 (21) 52380373 (400) 821-3475
FAX: +86 (21) 52371001
E-MAIL: info.cn@bio-techne.com

INTRODUCTION

Matrix MetalloProteinases (MMPs) are zinc-dependent proteases that catalyze degradation of extracellular matrix proteins, thereby controlling such processes as tissue morphogenesis, cell migration, wound healing, bone remodeling, angiogenesis, and tumor metastasis. MMP activities are modulated on several levels including transcription, pro-enzyme activation, or by their endogenous inhibitors, Tissue Inhibitors of MetalloProteinases (TIMPs) (1). TIMPs are small, secreted proteins that are involved in regulating MMPs during tissue remodeling by both inhibition of active MMPs and by activation of pro-MMPs (1,2). TIMPs inhibit MMPs by forming 1:1, non-covalent complexes with MMPs, thereby blocking access of substrates to the MMP catalytic site (2, 3). TIMPs are highly specific for MMPs in general but not for any particular MMP. While TIMPs share basic structural characteristics, they do have distinct biochemical features, expression patterns, and *in vivo* effects on cell growth, apoptosis, angiogenesis, and tumorigenesis (1-3). Many physiological functions of TIMPs are closely tied to the functions of MMPs, and an improper balance of MMP and TIMP production correlates with pathological conditions such as arthritis, cardiovascular disorders, tumor growth and metastasis (4). Expression levels of TIMPs may be valuable markers for carcinogenesis as expression is regulated in several cancer types and in some cases, correlates with stages of tumor malignancy or survival (5). In addition, TIMPs have activity that appears to be functionally distinct from MMP inhibitory activity. For example, TIMP-1 was independently discovered as a protein with erythroid-potentiating activity (6), while TIMP-2 suppresses EGF-mediated mitogenic signaling (7).

This kit can be used to simultaneously assess the levels of all four TIMP molecules in a single sample. For ease of use, the TIMP microparticles are pre-mixed in one vial and the biotinylated detection antibodies are pre-mixed as well.

Analyte	Bead Region
TIMP-1	12
TIMP-2	13
TIMP-3	14
TIMP-4	15

PRINCIPLE OF THE ASSAY

Magnetic Luminex® Performance Assay multiplex kits are designed for use with the Luminex® MAGPIX® CCD Imager. Alternatively, kits can be used with the Luminex® 100/200™, Luminex® FLEXMAP 3D®, or Bio-Rad® Bio-Plex®, dual laser, flow-based sorting and detection platforms.

Analyte-specific antibodies are pre-coated onto magnetic microparticles embedded with fluorophores at set ratios for each unique microparticle region. Microparticles, standards and samples are pipetted into wells and the immobilized antibodies bind the analytes of interest. After washing away any unbound substances, a biotinylated antibody cocktail specific to the analytes of interest is added to each well. Following a wash to remove any unbound biotinylated antibody, streptavidin-phycoerythrin conjugate (Streptavidin-PE), which binds to the biotinylated antibody, is added to each well. Final washes remove unbound Streptavidin-PE, the microparticles are resuspended in buffer and read using the Luminex® MAGPIX® Analyzer. A magnet in the analyzer captures and holds the superparamagnetic microparticles in a monolayer. Two spectrally distinct Light Emitting Diodes (LEDs) illuminate the microparticles. One LED excites the dyes inside each microparticle to identify the region and the second LED excites the PE to measure the amount of analyte bound to the microparticle. A sample from each well is imaged with a CCD camera with a set of filters to differentiate excitation levels.

Analysis with the Luminex® 100/200™, Luminex® FLEXMAP 3D®, or Bio-Rad Bio-Plex uses one laser to excite the dyes inside each microparticle to identify the microparticle region and the second laser to excite the PE to measure the amount of analyte bound to the microparticle. All excitation emitted as each microparticle passes through the flow cell is then analyzed to differentiate excitation levels using a Photomultiplier Tube (PMT) and an Avalanche Photodiode.

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 fall outside the dynamic range of the assay, further dilute the samples with the appropriate calibrator diluent and repeat the assay.
- Any variation in 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. Uuntil these factors have been tested in the Magnetic Luminex® Performance Assay, the possibility of interference cannot be excluded.
- Magnetic Luminex® Performance Assays afford the user the benefit of multianalyte analysis of biomarkers in a single complex sample. For each sample type, a single multipurpose diluent is used to optimize recovery, linearity, and reproducibility. Such a multipurpose diluent may not optimize any single analyte to the same degree that a unique diluent selected for analysis of that analyte can optimize conditions. Therefore, some performance characteristics may be more variable than those for assays designed specifically for single analyte analysis.
- **Only the analytes listed on the Standard Value Card can be measured with this kit.**

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, DILUTED, OR RECONSTITUTED MATERIAL
TIMP Standard Cocktail	893262	2 vials of recombinant human TIMPs in a buffered protein base with preservatives; lyophilized.	Use a fresh standard for each assay. Discard after use.
Human TIMP Panel Magnetic Microparticles	894429	0.60 mL of a concentrated human TIMP microparticle cocktail with preservatives.	May be stored for up to 1 month at 2-8 °C.* *Once diluted, 1X solutions must be discarded. Use fresh diluents for each assay.*
Human TIMP Biotin-Ab Concentrate	893261	0.60 mL of a concentrated human TIMP biotinylated antibody cocktail with preservatives.	
Streptavidin-PE	892525	0.07 mL of a concentrated streptavidin-phycoerythrin conjugate with preservatives.	
Microparticle Diluent 5	895575	6 mL of a buffered protein base with preservative.	May be stored for up to 1 month at 2-8 °C.*
Biotin Antibody Diluent 2	895832	5.5 mL of a buffered protein base with preservative.	
Calibrator Diluent RD6-48	895579	2 vials (21 mL/vial) of a buffered protein base with preservatives. *Use undiluted for serum/plasma/human milk samples. Use diluted 2:1 for cell culture supernate/ saliva/urine samples.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Microplate	641385	1 flat-bottomed 96-well microplate used as a vessel for the assay.	
Mixing Bottles	895505	2 empty 8 mL bottles used for mixing microparticles with Microparticle Diluent.	
Plate Sealers	640445	4 adhesive foil strips.	
Standard Value Card	749850	1 card listing the Standard reconstitution volume and working standard concentrations for this lot of standard.	

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

OTHER SUPPLIES REQUIRED

- Luminex® MAGPIX®, Luminex® 100/200™, Luminex® FLEXMAP 3D®, or Bio-Rad Bio-Plex analyzer with X-Y platform.
- Hand-held microplate magnet or platewasher with a magnetic platform.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Multi-channel pipette, manifold dispenser, or automated dispensing unit.
- 50 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 800 ± 50 rpm.
- Microcentrifuge.
- **Polypropylene** test tubes for dilution of standards and samples.
- Luminex® Performance Assay Control (optional; R&D Systems®, Catalog # QC16).

PRECAUTIONS

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

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

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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Protect microparticles and Streptavidin-PE from light at all times to prevent photobleaching.

SAMPLE COLLECTION AND 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 and 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 30 minutes at room temperature before centrifuging for 15 minutes at 1000 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 or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

Saliva - Collect saliva using a collection device such as a Salivette® or equivalent. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Saliva collector must not have any protein binding or filtering capabilities.*

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Human Milk - Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Use polypropylene tubes.

Note: *On the day of the assay, ALL fresh and previously frozen serum and plasma samples require centrifugation at 16,000 x g for 4 minutes immediately prior to use or dilution.*

Cell culture supernate samples require a 10-fold dilution. A suggested 10-fold dilution is 25 µL of sample + 225 µL of Calibrator Diluent RD6-48 (diluted 2:1)*. Mix thoroughly.

Serum/plasma samples require a 50-fold dilution. A suggested 50-fold dilution is 10 µL of sample + 490 µL of Calibrator Diluent RD6-48. Mix thoroughly.

Saliva samples require a 100-fold dilution. A suggested 100-fold dilution is 10 µL of sample + 990 µL of Calibrator Diluent RD6-48 (diluted 2:1)*. Mix thoroughly.

Urine samples require a 5-fold dilution. A suggested 5-fold dilution is 50 µL of sample + 200 µL of Calibrator Diluent RD6-48 (diluted 2:1)*. Mix thoroughly.

Human milk samples require a 200-fold dilution. A suggested 200-fold dilution is 10 µL of sample + 190 µL of Calibrator Diluent RD6-48. After this initial 20-fold dilution, combine 20 µL of the diluted sample + 180 µL of Calibrator Diluent RD6-48 for a final 200-fold dilution. Mix thoroughly.

*See Reagent Preparation section.

REAGENT PREPARATION

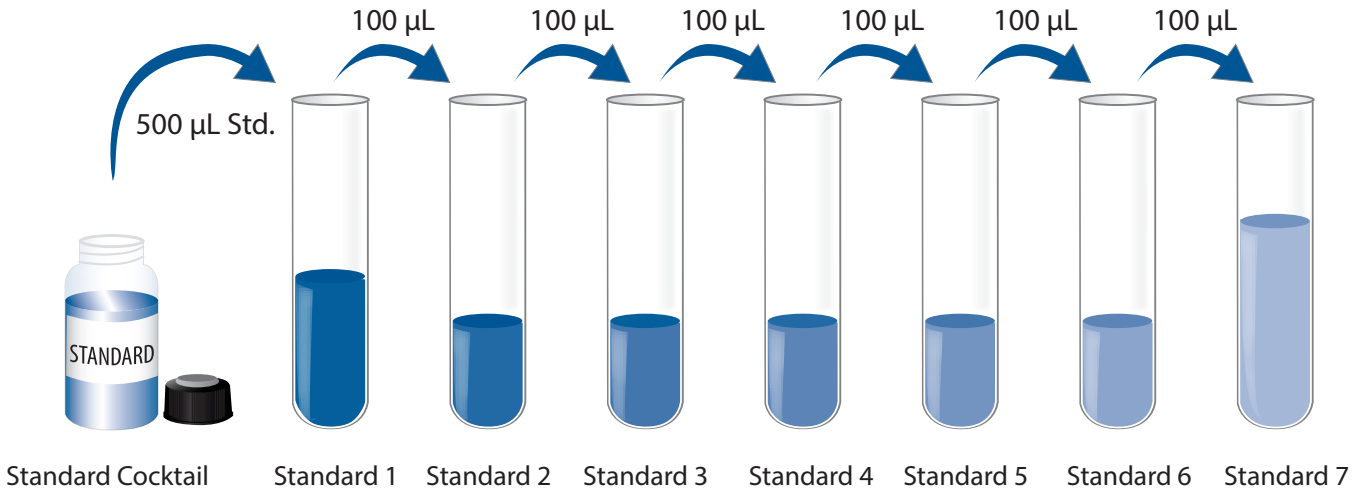
Bring all reagents to room temperature before use.

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 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Calibrator Diluent RD6-48 (diluted 2:1) - **For cell culture supernate/saliva/urine samples.** Add 20 mL of Calibrator Diluent RD6-48 to 10 mL of deionized or distilled water to prepare 30 mL of Calibrator Diluent RD6-48 (diluted 2:1).

Standard - **Refer to the Standard Value Card for the reconstitution volume and assigned values.** Reconstitute the Standard Cocktail with Calibrator Diluent RD6-48 (*for serum, plasma, and human milk samples*) or Calibrator Diluent RD6-48 (diluted 2:1) (*for cell culture supernate, saliva, and urine samples*). Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 500 µL of the reconstituted Standard Cocktail into the Standard 1 tube. Pipette 200 µL of the appropriate calibrator diluent into the remaining tubes. Use Standard 1 to produce a 3-fold dilution series (below). Mix each tube thoroughly before the next transfer. Standard 1 serves as the high standard. The appropriate calibrator diluent serves as the blank.



DILUTED MICROPARTICLE COCKTAIL PREPARATION

1. Centrifuge the Microparticle Cocktail vial for 30 seconds at 1000 x g prior to removing the cap.
2. Gently vortex the vial to resuspend the microparticles, taking precautions not to invert the vial.
3. Dilute the Microparticle Cocktail in the mixing bottle provided.

Number of Wells Used	Microparticle Cocktail	+	Microparticle Diluent
96	500 µL	+	5.0 mL
72	375 µL	+	3.75 mL
48	250 µL	+	2.5 mL
24	125 µL	+	1.25 mL

Note: *Protect microparticles from light during handling. Diluted microparticles cannot be stored. Prepare microparticles within 30 minutes of use.*

DILUTED BIOTIN-ANTIBODY COCKTAIL PREPARATION

1. Centrifuge the Biotin-Antibody vial for 30 seconds at 1000 x g prior to removing the cap.
2. Gently vortex the vial, taking precautions not to invert the vial.
3. Dilute the Biotin-Antibody Cocktail in Biotin Antibody Diluent 3. Mix gently.

Number of Wells Used	Biotin-Antibody Cocktail	+	Biotin Antibody Diluent 2
96	500 µL	+	5.0 mL
72	375 µL	+	3.75 mL
48	250 µL	+	2.5 mL
24	125 µL	+	1.25 mL

STREPTAVIDIN-PE PREPARATION

Use a polypropylene amber bottle or a polypropylene tube wrapped with aluminum foil. Protect Streptavidin-PE from light during handling and storage.

1. Centrifuge the Streptavidin-PE vial for 30 seconds at 1000 x g prior to removing the cap.
2. Gently vortex the vial, taking precautions not to invert the vial.
3. Dilute the Streptavidin-PE concentrate in Wash Buffer.

Number of Wells Used	Streptavidin-PE Concentrate	+	Wash Buffer
96	55.0 µL	+	5.50 mL
72	42.0 µL	+	4.10 mL
48	28.0 µL	+	2.75 mL
24	14.0 µL	+	1.35 mL

INSTRUMENT SETTINGS

Note: *Adjust the probe height setting on the analyzer to avoid puncturing the plate. Calibrate the analyzer using the proper reagents for superparamagnetic microparticles (refer to instrument manual).*

Luminex® MAGPIX® analyzer:

a) Sample volume: 50 µL

b) Assign the microparticle region for each analyte being measured (see page 1)

c) 50 count/region

d) Collect Median Fluorescence Intensity (MFI)

Luminex® 100/200™, Luminex® FLEXMAP 3D® and Bio-Rad Bio-Plex analyzers:

Note: *Ensure that the instrument flow rate is set to the default of 60 µL/minute (fast) for all flow based analyzers.*

a) Sample volume: 50 µL

b) Bead Type:

i. Luminex® 100/200™ and FLEXMAP 3D® select MagPlex

ii. Bio-Rad Bio-Plex Manager use Bio-Plex MagPlex Beads (Magnetic)

c) Doublet Discriminator gates:

i. Luminex® 100/200™ and FLEXMAP 3D® set at 8000 and 16,500

ii. Bio-Rad Bio-Plex Manager set at 8000 and 23,000

d) Reporter Gain Setting:

i. Luminex® 100/200™ use Default setting

ii. Luminex® FLEXMAP 3D® use Standard PMT setting

iii. Bio-Rad Bio-Plex Manager use the low RP1 target value for the CAL2 setting

e) Assign the microparticle region for each analyte being measured (see page 1)

f) 50 count/region

g) Collect MFI

ASSAY PROCEDURE

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

Note: *Protect microparticles and Streptavidin-PE from light at all times.*

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Add 50 µL of standard, control, or sample* per well. A plate layout is provided to record standards and samples assayed.
3. Resuspend the diluted Microparticle Cocktail by inversion or vortexing. Add 50 µL of the Microparticle Cocktail to each well of the microplate. Securely cover with a foil plate sealer. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 800 ± 50 rpm.
4. Using a magnetic device designed to accommodate a microplate, wash by applying the magnet to the bottom of the microplate, allow 1 minute before removing the liquid, filling each well with Wash Buffer (100 µL) and allow 1 minute before removing the liquid again. Complete removal of liquid is essential for good performance. **Note:** ***Do NOT blot; this may cause a loss of microparticles.*** Perform the wash procedure three times.

   Note: *Refer to the magnetic device user manual for proper wash technique using a round bottom microplate.*
5. Add 50 µL of diluted Biotin-Antibody Cocktail to all wells. Securely cover with a foil plate sealer and incubate for 1 hour at room temperature on the shaker set at 800 ± 50 rpm.
6. Repeat the wash as in step 4.
7. Add 50 µL of diluted Streptavidin-PE to all wells. Securely cover with a foil plate sealer and incubate for 30 minutes at room temperature on the shaker set at 800 ± 50 rpm.
8. Repeat the wash as in step 4.
9. Resuspend the microparticles by adding 100 µL of Wash Buffer to each well. Incubate for 2 minutes on the shaker set at 800 ± 50 rpm.
10. Read within 90 minutes using the Luminex® or BioRad Analyzer.
    Note: *Resuspend microparticles immediately prior to reading by shaking the plate for 2 minutes on the plate shaker at 800 ± 50 rpm.*

*Samples require dilution. See Sample Preparation section.

ASSAY PROCEDURE SUMMARY

Note: *Protect microparticles and Streptavidin-PE from light at all times.*

❶ Prepare all reagents as instructed.

❷ Add 50 µL of standard, control, or sample* to each well.

❸ Add 50 µL of diluted Microparticle Cocktail to each well.
Incubate for 2 hours at RT on a shaker at 800 rpm.

❹ Wash by removing the liquid from each well, filling with 100 µL Wash Buffer, and removing the liquid again.
Perform the wash 3 times.

❺ Add 50 µL of diluted Biotin-Antibody Cocktail to each well.
Cover and incubate for 1 hour at RT on the shaker at 800 rpm.

❻ Repeat the wash as in step 4.

❼ Add 50 µL of diluted Streptavidin-PE to each well.
Incubate for 30 minutes at RT on the shaker at 800 rpm.

❽ Repeat the wash as in step 4.

❾ Add 100 µL of Wash Buffer to each well.
Incubate for 2 minutes at RT on the shaker at 800 rpm.

❿ Read within 90 minutes using a Luminex® or Bio-Rad analyzer
Note: *Resuspend microparticles immediately prior to reading.*

*Samples require dilution. See Sample Preparation sections.

CALCULATION OF RESULTS

Use the Standard concentrations on the Standard Value Card and calculate 3-fold dilutions for the remaining levels. Average the duplicate readings for each standard and sample and subtract the average blank Median Fluorescence Intensity (MFI).

Create a standard curve for each analyte by reducing the data using computer software capable of generating a five parameter logistic (5-PL) curve-fit.

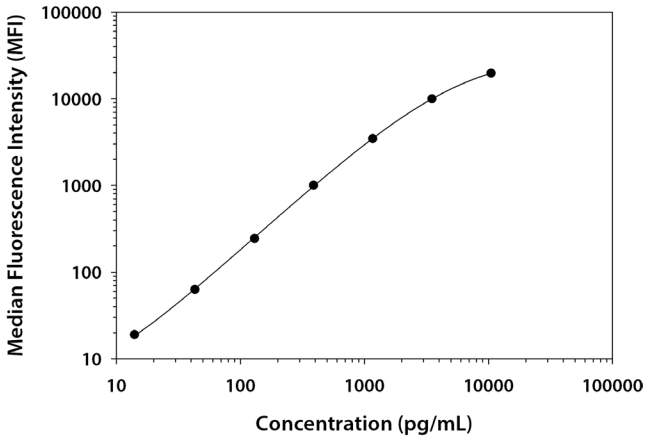
Since samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

These standard curves are provided only for demonstration. Standard curves must be generated each time an assay is run, utilizing values from the included Standard Value Card.

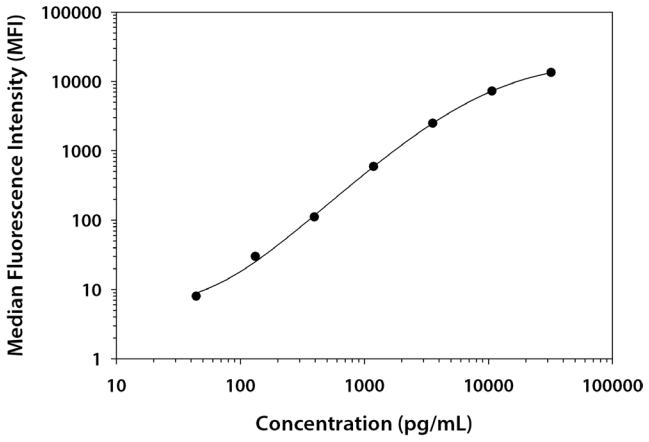
Note: TIMP-3 *utilizes a six point standard curve. When fitting a standard curve constructed with the recommended 3-fold dilution series, only use the first six points (omit the lowest concentration standard).*

TIMP-1



Standard	(pg/mL)	MFI	Average	Corrected
Blank	0	17 18	18	—
Standard 1	10,500	19,592 19,850	19,721	19,703
Standard 2	3500	9947 10,003	9975	9957
Standard 3	1167	3367 3582	3475	3457
Standard 4	389	996 1045	1021	1003
Standard 5	130	255 267	261	243
Standard 6	43	77 83	80	62
Standard 7	14	36 36	36	18

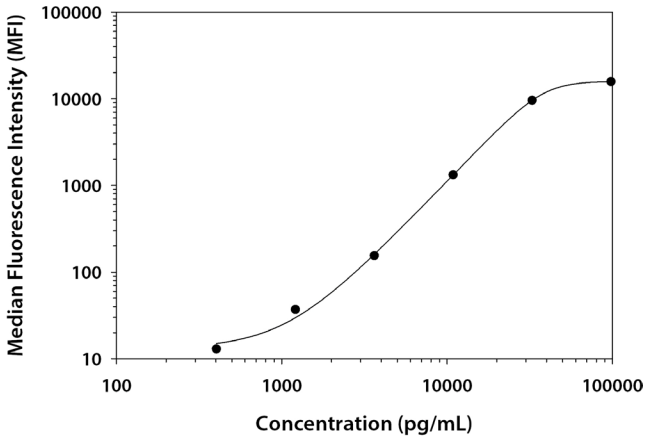
TIMP-2



Standard	(pg/mL)	MFI	Average	Corrected
Blank	0	21 22	22	—
Standard 1	32,000	13,337 13,555	13,446	13,424
Standard 2	10,667	7203 7339	7271	7249
Standard 3	3556	2439 2587	2513	2491
Standard 4	1185	604 621	613	591
Standard 5	395	128 136	132	110
Standard 6	132	46 56	51	29
Standard 7	44	29 30	30	8

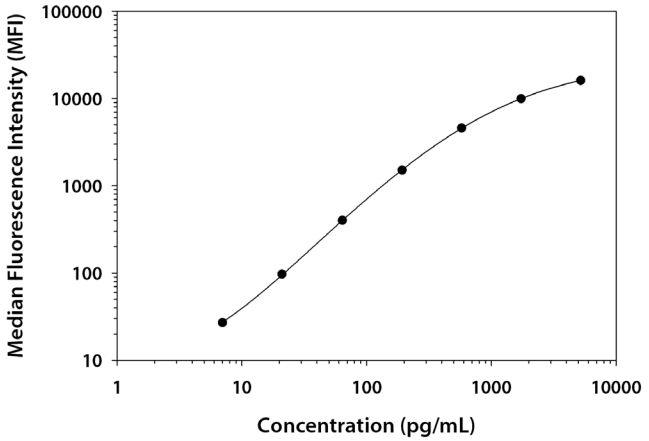
TYPICAL DATA *CONTINUED*

TIMP-3



Standard	(pg/mL)	MFI	Average	Corrected
Blank	0	15 15	15	—
Standard 1	98,000	15,767 15,767	15,767	15,752
Standard 2	32,667	9583 9584	9584	9569
Standard 3	10,889	1310 1367	1339	1324
Standard 4	3630	170 170	170	155
Standard 5	1210	51 53	52	37
Standard 6	403	27 28	28	13

TIMP-4



Standard	(pg/mL)	MFI	Average	Corrected
Blank	0	18 18	18	—
Standard 1	5200	15,955 16,230	16,093	16,075
Standard 2	1733	9897 10,014	9956	9938
Standard 3	578	4520 4679	4600	4582
Standard 4	193	1490 1565	1528	1510
Standard 5	64	414 424	419	401
Standard 6	21	115 115	115	97
Standard 7	7	45 45	45	27

CALIBRATION

This assay is calibrated against highly purified recombinant human TIMPs produced at R&D Systems®.

PERFORMANCE CHARACTERISTICS

Data obtained with polystyrene and magnetic beads were equivalent.

SENSITIVITY

Forty-one assay were run and the minimum detectable dose (MDD) was determined by adding two standard deviations to the MFI of twenty zero standard replicates and calculating the corresponding concentration.

Analyte	Mean (pg/mL)	Range (pg/mL)
TIMP-1	1.54	0.6-3.43
TIMP-2	14.7	4.6-40.1
TIMP-3	86	20-253
TIMP-4	1.29	0.28-3.80

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 forty-eight separate assays to assess inter-assay precision.

TIMP-1

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	48	48	48
Mean (pg/mL)	40	395	3242	47	359	3078
Standard deviation	4.0	18	210	7.2	36	257
CV (%)	10.0	4.6	6.5	15.3	10.0	8.3

TIMP-2

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	48	48	48
Mean (pg/mL)	562	3538	13,046	577	3146	13,483
Standard deviation	29	119	543	76	260	1089
CV (%)	5.2	3.4	4.2	13.2	8.3	8.1

TIMP-3

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	48	48	48
Mean (pg/mL)	2416	15,047	29,412	2029	15,944	29,298
Standard deviation	145	733	627	316	1550	2644
CV (%)	6.0	4.9	2.1	15.6	9.7	9.0

TIMP-4

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	48	48	48
Mean (pg/mL)	36	126	1239	37	121	1219
Standard deviation	2.3	5.0	86	4.9	12	114
CV (%)	6.4	4.0	6.9	13.2	9.9	9.4

RECOVERY

The recovery of TIMPs spiked to levels throughout the range of the assay in various matrices was evaluated.

TIMP-1

Sample Type	Average % Recovery	Range
Cell culture supernates	101	64-126%
Urine	92	66-121%

TIMP-2

Sample Type	Average % Recovery	Range
Cell culture supernates	94	62-128%
Serum	84	65-118%
EDTA plasma	82	52-120%
Heparin plasma	83	69-104%
Urine	85	59-118%

TIMP-3

Sample Type	Average % Recovery	Range
Cell culture supernates	106	60-133%
Serum	93	76-122%
EDTA plasma	96	85-128%
Heparin plasma	108	96-140%
Urine	104	82-126%

TIMP-4

Sample Type	Average % Recovery	Range
Cell culture supernates	100	82-119%
Serum	94	85-105%
EDTA plasma	92	83-103%
Heparin plasma	91	75-109%
Urine	99	72-124%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of TIMPs were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

TIMP-1

		Cell culture supernates	Serum	EDTA plasma	Heparin plasma	Urine
1:2	Average % of Expected	96	106	104	105	101
	Range (%)	78-115	94-124	96-120	96-120	83-122
1:4	Average % of Expected	95	112	105	105	103
	Range (%)	80-119	98-125	96-120	95-115	79-123
1:8	Average % of Expected	92	115	103	104	101
	Range (%)	72-112	95-137	90-118	88-117	82-129

TIMP-2

		Cell culture supernates	Serum	EDTA plasma	Heparin plasma	Urine
1:2	Average % of Expected	103	96	94	97	105
	Range (%)	81-128	83-108	86-100	84-113	89-127
1:4	Average % of Expected	106	102	100	93	107
	Range (%)	76-131	88-116	92-108	66-122	92-131
1:8	Average % of Expected	102	103	102	94	104
	Range (%)	53-128	89-119	94-113	71-122	84-123

TIMP-3

		Cell culture supernates	Serum	EDTA plasma	Heparin plasma	Urine
1:2	Average % of Expected	100	91	86	86	91
	Range (%)	84-116	78-111	71-108	78-109	79-109
1:4	Average % of Expected	96	84	78	75	83
	Range (%)	69-129	72-108	65-102	66-98	75-103
1:8	Average % of Expected	88	83	79	74	80
	Range (%)	65-123	70-102	70-100	64-93	68-102

TIMP-4

		Cell culture supernates	Serum	EDTA plasma	Heparin plasma	Urine
1:2	Average % of Expected	98	91	89	90	98
	Range (%)	82-120	79-109	74-102	78-101	76-115
1:4	Average % of Expected	94	88	89	90	96
	Range (%)	78-115	74-103	79-100	79-107	79-106
1:8	Average % of Expected	88	85	86	86	93
	Range (%)	67-109	73-98	73-101	73-96	79-109

SPECIFICITY

This assay recognizes natural and recombinant human TIMPs.

Cross-reactivity - The factors listed below were prepared at 200 ng/mL in calibrator diluent, assayed, and measured less than 0.5% cross-reactivity unless otherwise noted.

Recombinant human:

6Ckine
ADAM9
ADAM10
ADAM15
ADAM33
ADAMTS1
ADAMTSL-1
ADAMTSL-2
Lipocalin-1
Lipocalin-2
MMP-1
MMP-2*
MMP-3
MMP-7
MMP-9
MMP-9/TIMP-1 complex*
MMP-10
MMP-12
MMP-13
MMP-14
MMP-16
TACE (ADAM17)

Recombinant mouse:

ADAM9
ADAM10
ADAM15
Lipocalin-2
MMP-2
MMP-3
MMP-7
MMP-9
MMP-12
TACE
TIMP-1
TIMP-2

Recombinant rat:

TIMP-1

*Recombinant human MMP-9/TIMP-1 complex cross-reacts approximately 2.39% with TIMP-1 and recombinant human MMP-2 cross-reacts approximately 0.6% with TIMP-2.

SPECIFICITY *CONTINUED*

Interference - Preparations of the factors on the previous page were prepared at 200 ng/mL in a mid-range recombinant human TIMP standard and assayed for interference. The following factors interfered:

TIMP-1

Recombinant Factor	Concentration (ng/mL)
Human MMP-9	> 66.67

TIMP-2

Recombinant Factor	Concentration (ng/mL)
Human MMP-9/TIMP-1	> 66.67
Mouse MMP-2	> 0.823

TIMP-3

Recombinant Factor	Concentration (ng/mL)
Human MMP-3	> 66.67
Human MMP-7	> 66.67
Human TACE	> 22.22
Mouse MMP-12	> 22.22
Mouse TACE	> 22.22

TIMP-4

Recombinant Factor	Concentration (ng/mL)
Human TACE	> 22.22
Mouse ADAM10	> 66.67

Additionally, none of the multiplex partners showed cross-reactivity or interference in the pre-mixed microparticles or biotin-antibodies.

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.