Mosaic™ ELISA

Human MMP Panel

Catalog Number MEA006

For the simultaneous quantitative determination of concentrations of multiple human Matrix Metalloproteinases (MMPs) in cell culture supernates, serum, plasma, saliva, and urine.

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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INTRODUCTION

Matrix metalloproteinases (MMPs) are a family of zinc and calcium dependent endopeptidases that are responsible for the degradation of all the components of the extracellular matrix. MMPs contribute to both normal and pathological tissue remodeling, and play key roles in the migration of normal and malignant cells through the body(1-2). They also act as regulatory molecules, both by functioning in enzyme cascades and by processing matrix proteins, cytokines, growth factors, and adhesion molecules to generate fragments with enhanced or reduced biological effects. The Human MMP Mosaic ELISA is an excellent tool for the detection of 7 different MMPs in the same sample. Mosaic kits employ multiplex microarray technology to provide an accurate, efficient, and economical alternative to conducting multiple traditional ELISA experiments.

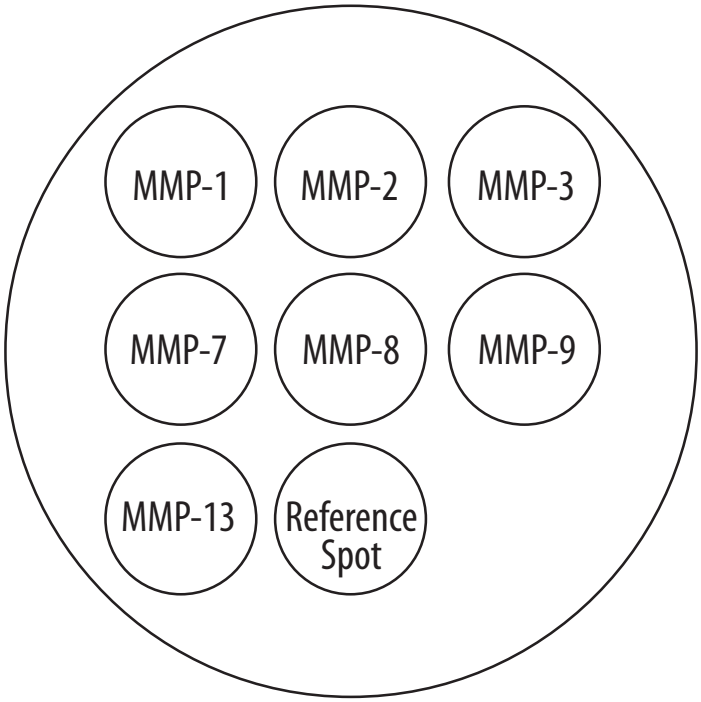


Figure 1: A visualization of the spot layout per well.

PRINCIPLE OF ASSAY

The Mosaic Human MMP Panel Immunoassay employs a two-site sandwich ELISA technique to simultaneously detect 7 MMPs in cell culture supernates, serum, plasma, saliva, and urine. Multiple capture antibodies that specifically recognize the target MMPs have been pre-spotted into each well of a 96 well microplate. Standards and samples are added, and MMPs present in the samples are bound by the immobilized antibodies. After washing away unbound material, biotinylated detection antibodies are used to detect the specific MMPs. Unbound detection antibodies are washed away and streptavidin-HRP is added. Following an additional wash, chemiluminescent substrate reagents are added to the wells, and a signal proportional to the amount of each protein biomarker bound in the initial step is produced. Plates are read using a digital camera imaging system, and pixel intensity is measured using an analytical software package.

TECHNICAL HINTS AND LIMITATION

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- This 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.
- Any variation in buffers, operator, pipetting technique, washing technique, instrumentation, and incubation time or temperature and kit age can alter the performance of the kit.
- Variations in sample collection, processing, and storage may cause sample value differences.
- 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.
- Avoid microbial contamination of reagents and buffers.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- If samples fall outside the dynamic range of the assay, further dilute the samples with Calibrator Diluent and repeat the assay.
- Mosaic affords the user the benefit of multianalyte analysis of 7 MMPs in a complex sample. A multipurpose diluent for each sample type 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.
- This assay is designed to eliminate interference by other enzymes and proteins present in biological samples. Until all factors have been tested in the Mosaic assay, the possibility of interference cannot be excluded.
- Discrepancies may exist in values obtained for the same analyte utilizing different technologies.
- **Only the analytes listed in Figure 1 (or on the enclosed Standard Value Card) can be measured with this kit.**

PRECAUTION

MMPs are detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

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
Microplate	893598	96 well microplate spotted with 7 antibodies against specific MMPs.	Invert the plate, and blot it against clean paper towels to dry the plate. Return it to the foil pouch containing the desiccant pack, and reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*
Standard	893599	2 vials of a cocktail of recombinant human MMPs in a buffered protein base with preservatives; lyophilized.	Discard after use. Use a fresh standard for each assay.
Detection Mix	895965	6 mL of a cocktail of antibodies conjugated to biotin with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD2-1	895970	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-37	895853	21 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	2 vials (21 mL/vial) of a 25-fold concentrated solution of buffered surfactant with preservatives.	
Streptavidin-HRP	895469	6 mL of a streptavidin-horseradish peroxidase conjugate with preservatives.	
Substrate 1	895471	3 mL of a buffered solution.	
Substrate 2	895472	3 mL of a buffered solution.	
Plate Sealers	N/A	8 adhesive strips.	Store at room temperature.
Standard Value Card	749984	1 card listing the standard reconstitution volume and concentrations for this lot of standard.	

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

OTHER SUPPLIES REQUIRED

- Pipettes and pipette tips.
- Deionized or distilled water.
- Manifold dispenser, squirt bottle, or automated microplate washer.
- Graduated cylinders for preparing Wash Buffer.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Digital Imaging System (for details, visit www.RnDSystems.com/go/ImagingSystems).
- **Polypropylene** test tubes for dilution of standards and samples.

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

Serum - Allow blood 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 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.

Platelet-Poor Plasma - Collect plasma on ice using heparin as an anticoagulant. Centrifuge at 2-8 °C at 1000 x g within 30 minutes of collection. An additional centrifugation step of the separated plasma at 10,000 x g for 10 minutes is recommended for complete platelet removal. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *EDTA and Citrate plasma are not recommended anticoagulants for use in this assay due to their chelating properties.*
Grossly hemolyzed and/or lipemic samples are not suitable for use in this assay.

Some MMPs may be released upon platelet activation. For example, to measure circulating levels of MMP-9, platelet-poor plasma should be used. It should be noted that many protocols for plasma preparation, including procedures recommended by the National Committee for Clinical Laboratory Standards (NCCLS), result in incomplete removal of platelets or platelet activation. This may cause variable and irreproducible results for assays of factors contained in platelets and released by platelet activation.

Saliva - Collect saliva in a tube and centrifuge for 5 minutes at 10,000 x g. Collect the aqueous layer, and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, and assay immediately or aliquot and store at ≤ -70 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Cell culture supernate samples require at least a 5-fold dilution. A suggested 5-fold dilution is 40 µL of sample + 160 µL of Calibrator Diluent RD5-37. Mix thoroughly.

Serum/plasma/platelet-poor plasma samples require at least a 10-fold dilution. A suggested 10-fold dilution is 15 µL of sample + 135 µL of Calibrator Diluent RD5-37. Mix thoroughly.

Note: *When assaying MMP-9, serum and platelet-poor plasma samples must be further diluted 10-fold to a final 100-fold dilution. A suggested 100-fold dilution is 15 µL of the 10-fold diluted sample + 135 µL of Calibrator Diluent RD5-37. Mix thoroughly.*

Saliva samples require at least a 40-fold dilution. A suggested 40-fold dilution is 10 µL of sample + 390 µL of Calibrator Diluent RD5-37.

Urine samples require at least a 10-fold dilution. A suggested 10-fold dilution is 15 µL of sample + 135 µL of Calibrator Diluent RD5-37.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

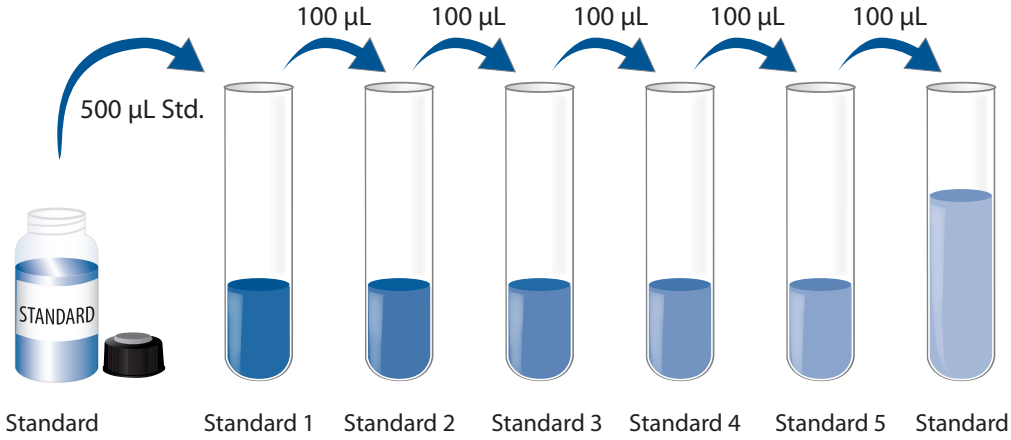
Note: *MMPs are detectable in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

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

Substrate Solution - Substrates 1 and 2 should be mixed together in equal volumes 2-30 minutes prior to use. Protect from light. 50 µL of the resultant mixture is required per well.

Standard - Reconstitute the Standard Cocktail with Calibrator Diluent RD5-37. Refer to the Standard Value Card for the reconstitution volume and assigned values. 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 into the Standard 1 tube. Pipette 200 µL of 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. Calibrator Diluent RD5-37 serves as the zero standard.



ASSAY PROCEDURE

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

Note: *Protect Streptavidin-HRP and the Substrate from light at all times.*
MMPs are detectable in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Add 50 µL of Assay Diluent RD2-1 to each well.
3. Add 50 µL of Standard or sample* per well. Securely cover with a plate sealer. Incubate for 3 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout is provided as a record of standards and samples assayed.
4. Aspirate each well and wash, repeating the process three times for a total of four 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.
5. Add 50 µL of the Detection Mix to all wells. Securely cover with a plate sealer and incubate for 1 hour at room temperature on the shaker set at 500 ± 50 rpm.
6. Repeat the wash as in step 4.
7. Add 50 µL of Streptavidin-HRP to all wells. Securely cover with a plate sealer and incubate for 30 minutes at room temperature on the shaker set at 500 ± 50 rpm.
8. Repeat the wash as in step 4.
9. Add 50 µL of Substrate Solution to each well.
10. Place the microplate in the imager. Wait no longer than 15 minutes to commence imaging.
    Note: *For details, visit www.RnDSystems.com/go/ImagingSystems.*

*Samples require dilution. See the Sample Preparation section.

INSTRUMENTATION

The Mosaic ELISA Kits have been validated on the Q-View™ Imager from Quansys Biosciences. Please visit **www.RnDSystems.com/go/ImagingSystems** for suitable imaging systems and their instructions for use.

SENSITIVITY

Twenty-seven assays were evaluated and the minimum detectable dose (MDD) was determined by adding two standard deviations to the mean pixel intensity of twenty zero standard replicates and calculating the corresponding concentration.

Analyte	Mean (pg/mL)	Range (pg/mL)
MMP-1	28.0	10.9-41.7
MMP-2	120	41.0-208
MMP-3	4.11	2.09-6.11
MMP-7	2.23	1.20-4.49
MMP-8	21.6	11.6-51.3
MMP-9	44.7	21.2-93.9
MMP-13	36.1	18.5-80.4

CALIBRATION

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

CALCULATION OF RESULTS

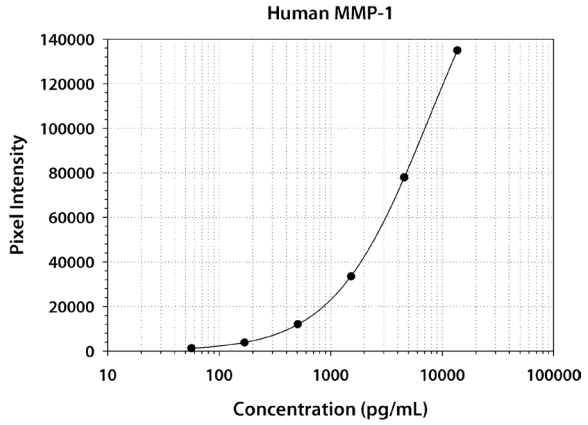
Use the Standard concentrations on the Standard Value Card and calculate 3-fold dilutions for the remaining levels. Average the duplicate readings for each standard and sample and subtract the average zero standard median pixel intensity (PI).

Create a standard curve for each analyte by reducing the data using computer software capable of generating a 5-PL curve fit. As an alternative, construct a standard curve by plotting the median PI 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 concentrations versus the log of the PI and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

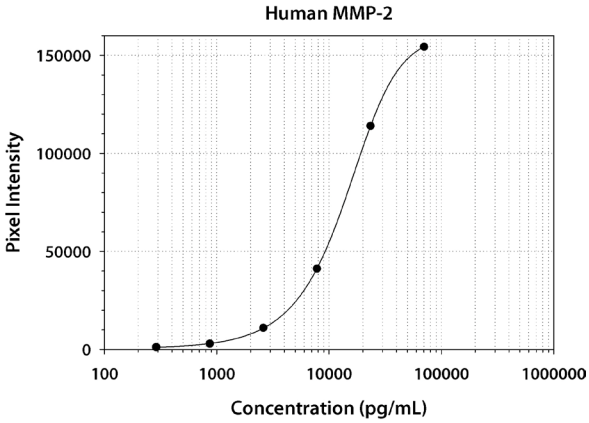
Since samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

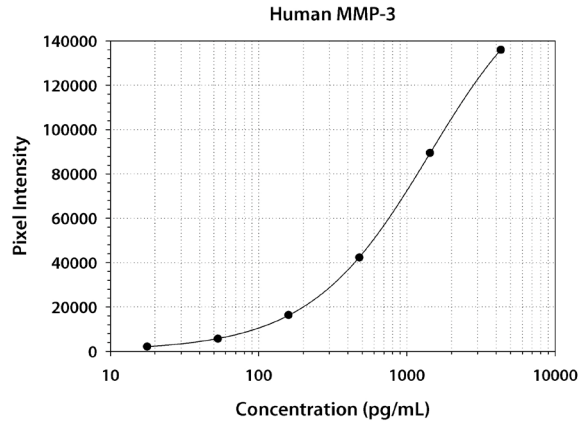
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



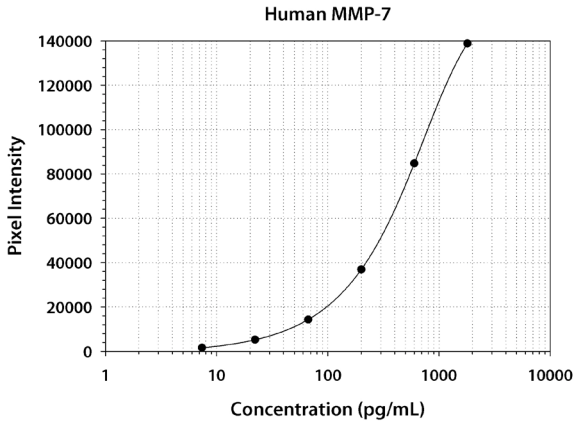
Standard	(pg/mL)	PI	Average	Corrected
Blank	0	2445 2495	2470	—
Standard 1	13,700	137,153 137,757	137,455	134,985
Standard 2	4567	80,365 80,517	80,441	77,971
Standard 3	1522	35,395 36,478	35,937	33,467
Standard 4	507	14,459 14,558	14,509	12,039
Standard 5	169	6198 6292	6245	3775
Standard 6	56.4	3650 3790	3720	1250



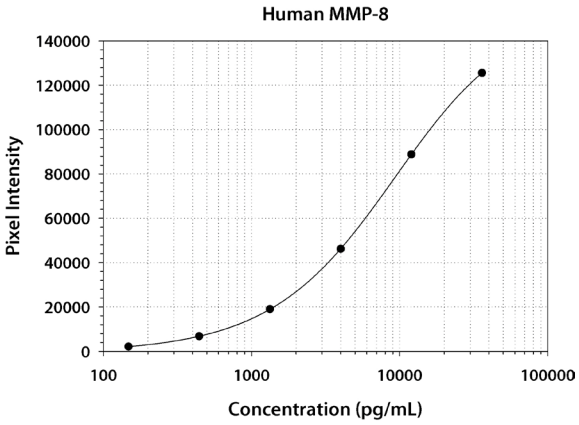
Standard	(pg/mL)	PI	Average	Corrected
Blank	0	1714 1751	1733	—
Standard 1	70,200	155,029 157,189	156,109	154,376
Standard 2	23,400	115,588 115,873	115,731	113,998
Standard 3	7800	42,461 43,461	42,961	41,228
Standard 4	2600	12,595 12,918	12,757	11,024
Standard 5	867	4662 4778	4720	2987
Standard 6	289	2780 3102	2941	1208



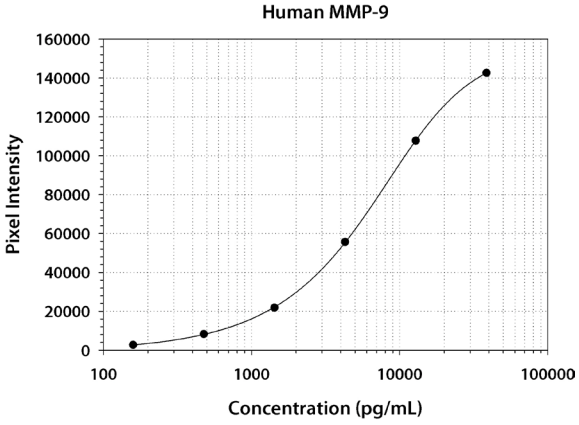
Standard	(pg/mL)	PI	Average	Corrected
Blank	0	1741 1742	1742	—
Standard 1	4300	137,675 137,717	137,696	135,954
Standard 2	1433	90,888 91,516	91,202	89,460
Standard 3	478	43,277 44,801	44,039	42,297
Standard 4	159	18,099 18,102	18,101	16,359
Standard 5	53.1	7262 7565	7414	5672
Standard 6	17.7	3772 3947	3860	2118



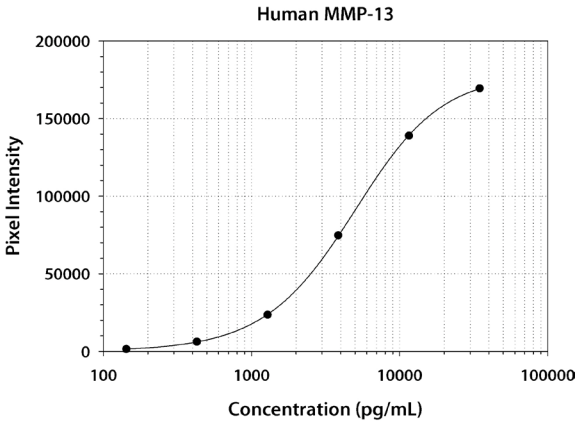
Standard	(pg/mL)	PI	Average	Corrected
Blank	0	1896 1970	1933	—
Standard 1	1800	140,470 141,011	140,741	138,808
Standard 2	600	85,831 87,542	86,687	84,754
Standard 3	200	38,523 39,137	38,830	36,897
Standard 4	66.7	16,026 16,419	16,223	14,290
Standard 5	22.2	6853 7446	7150	5217
Standard 6	7.40	3256 3777	3517	1584



Standard	(pg/mL)	PI	Average	Corrected
Blank	0	1346 1370	1358	—
Standard 1	36,000	126,725 127,090	126,908	125,550
Standard 2	12,000	89,698 90,636	90,167	88,809
Standard 3	4000	45,499 49,582	47,541	46,183
Standard 4	1333	20,234 20,524	20,379	19,021
Standard 5	444	7988 8278	8133	6775
Standard 6	148	3315 3634	3475	2117



Standard	(pg/mL)	PI	Average	Corrected
Blank	0	2498 2530	2514	—
Standard 1	38,600	144,813 145,320	145,067	142,553
Standard 2	12,867	109,445 111,059	110,252	107,738
Standard 3	4289	57,584 58,742	58,163	55,649
Standard 4	1430	24,187 24,529	24,358	21,844
Standard 5	477	10,713 10,824	10,769	8255
Standard 6	159	5079 5254	5167	2653



Standard	(pg/mL)	PI	Average	Corrected
Blank	0	1726 1756	1741	—
Standard 1	34,700	170,733 171,514	171,124	169,383
Standard 2	11,567	139,468 141,737	140,603	138,862
Standard 3	3856	75,697 77,165	76,431	74,690
Standard 4	1285	22,839 27,617	25,228	23,487
Standard 5	428	7857 7927	7892	6151
Standard 6	143	3111 3175	3143	1402

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 seventy-six separate assays to assess inter-assay precision.

MMP-1

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	76	76	76
Mean (pg/mL)	165	1220	5509	169	1311	6307
Standard deviation	14.7	87.6	261	22.5	154	651
CV (%)	8.9	7.2	4.7	13.3	11.7	10.3

MMP-2

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	76	76	76
Mean (pg/mL)	1417	10,113	35,607	1317	11,026	47,516
Standard deviation	104	439	1521	184	1642	6280
CV (%)	7.3	4.3	4.3	14.0	14.9	13.2

MMP-3

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	76	76	76
Mean (pg/mL)	58.7	502	2230	63.3	503	2241
Standard deviation	4.4	33.3	123	7.9	51.6	181
CV (%)	7.5	6.6	5.5	12.5	10.3	8.1

MMP-7

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	76	76	76
Mean (pg/mL)	20.8	169	616	22.0	160	646
Standard deviation	2.0	13.4	28.3	2.5	16.6	48.7
CV (%)	9.6	7.9	4.6	11.4	10.4	7.5

MMP-8

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	76	76	76
Mean (pg/mL)	530	3876	9709	567	3863	9086
Standard deviation	25.1	345	590	63.6	412	680
CV (%)	4.7	8.9	6.1	11.1	10.7	7.5

MMP-9

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	76	76	76
Mean (pg/mL)	770	4978	20,067	757	4802	20,762
Standard deviation	35.1	352	1152	95.4	577	2041
CV (%)	4.6	7.1	5.7	12.6	12.0	9.8

MMP-13

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	76	76	76
Mean (pg/mL)	395	6201	19,028	389	5155	21,617
Standard deviation	24.6	392	1458	57.0	704	3061
CV (%)	6.2	6.3	7.7	14.7	13.6	14.2

RECOVERY

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

Note: *Samples were diluted prior to assay as described in the Sample Preparation section.*

MMP-1

Sample Type	Average % Recovery	Range
Cell culture supernates	111	96-122%
Serum	99	89-106%
Heparin plasma	104	95-112%
Platelet-poor heparin plasma	115	94-125%
Urine	103	87-116%

MMP-2

Sample Type	Average % Recovery	Range
Cell culture supernates	119	101-133%
Serum	99	90-109%
Heparin plasma	96	80-107%
Platelet-poor heparin plasma	104	83-130%
Urine	105	72-130%

MMP-3

Sample Type	Average % Recovery	Range
Cell culture supernates	104	85-116%
Serum	91	83-100%
Heparin plasma	98	84-125%
Platelet-poor heparin plasma	104	91-124%
Urine	90	75-104%

MMP-7

Sample Type	Average % Recovery	Range
Cell culture supernates	104	91-118%
Serum	100	85-112%
Heparin plasma	105	90-128%
Platelet-poor heparin plasma	103	81-115%
Urine	86	70-101%

MMP-8

Sample Type	Average % Recovery	Range
Cell culture supernates	108	90-119%
Serum	87	73-99%
Heparin plasma	85	74-95%
Platelet-poor heparin plasma	103	86-114%
Urine	102	80-118%

MMP-9

Sample Type	Average % Recovery	Range
Cell culture supernates	84	76-93%
Serum	97	86-114%
Heparin plasma	NR	NR
Platelet-poor heparin plasma	93	74-123%
Urine	94	70-115%

NR=Not Recommended

MMP-13

Sample Type	Average % Recovery	Range
Cell culture supernates	109	91-127%
Serum	93	82-109%
Heparin plasma	107	88-118%
Platelet-poor heparin plasma	109	70-124%
Urine	104	87-116%

LINEARITY

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

Note: *Samples were diluted prior to assay as described in the Sample Preparation section.*

MMP-1

		Cell culture supernates	Serum	Heparin plasma	Platelet-poor heparin plasma	Saliva	Urine
1:2	Average % of Expected	105	102	95	112	———	101
	Range (%)	90-119	92-108	90-100	109-116	———	98-106
1:4	Average % of Expected	99	109	91	96	———	102
	Range (%)	85-121	103-115	85-94	95-99	———	94-108
1:8	Average % of Expected	87	108	92	94	———	106
	Range (%)	86-89	97-114	82-98	91-98	———	98-117

MMP-2

		Cell culture supernates	Serum	Heparin plasma	Platelet-poor heparin plasma	Saliva	Urine
1:2	Average % of Expected	106	119	122	104	———	93
	Range (%)	85-117	109-132	108-134	93-117	———	84-100
1:4	Average % of Expected	91	109	111	96	———	86
	Range (%)	69-107	95-118	97-127	81-102	———	77-94
1:8	Average % of Expected	80	102	88	90	———	79
	Range (%)	69-90	91-114	80-104	78-107	———	73-84

MMP-3

		Cell culture supernates	Serum	Heparin plasma	Platelet-poor heparin plasma	Saliva	Urine
1:2	Average % of Expected	101	100	101	98	———	101
	Range (%)	94-115	96-102	98-104	96-101	———	98-105
1:4	Average % of Expected	98	100	102	101	———	103
	Range (%)	95-104	98-101	96-104	95-106	———	99-110
1:8	Average % of Expected	99	100	99	99	———	102
	Range (%)	92-104	97-103	96-103	95-104	———	92-114

MMP-7

		Cell culture supernates	Serum	Heparin plasma	Platelet-poor heparin plasma	Saliva	Urine
1:2	Average % of Expected	103	102	102	97	99	100
	Range (%)	101-105	100-103	95-106	92-101	96-104	95-109
1:4	Average % of Expected	100	100	102	97	103	98
	Range (%)	94-104	98-103	102-104	93-101	97-108	92-109
1:8	Average % of Expected	93	95	99	92	105	94
	Range (%)	85-101	87-100	95-105	88-99	97-119	88-105

MMP-8

		Cell culture supernates	Serum	Heparin plasma	Platelet-poor heparin plasma	Saliva	Urine
1:2	Average % of Expected	94	109	104	99	100	91
	Range (%)	88-101	102-115	93-113	95-104	92-104	84-103
1:4	Average % of Expected	91	115	111	114	101	86
	Range (%)	81-101	104-121	99-120	107-120	96-105	78-95
1:8	Average % of Expected	89	120	113	114	99	84
	Range (%)	89-90	108-130	98-122	106-123	95-104	72-90

MMP-9

		Cell culture supernates	Serum	Heparin plasma	Platelet-poor heparin plasma	Saliva	Urine
1:2	Average % of Expected	110	112	NR	116	110	89
	Range (%)	103-121	106-120	NR	110-123	107-112	81-95
1:4	Average % of Expected	116	115	NR	116	115	86
	Range (%)	111-119	103-122	NR	106-126	110-120	75-92
1:8	Average % of Expected	114	120	NR	112	112	85
	Range (%)	101-126	95-134	NR	106-124	104-119	76-94

NR=Not Recommended

MMP-13

		Cell culture supernates	Serum	Heparin plasma	Platelet-poor heparin plasma	Saliva	Urine
1:2	Average % of Expected	76	112	98	101	———	103
	Range (%)	72-80	105-120	95-100	90-110	———	97-107
1:4	Average % of Expected	93	118	95	91	———	96
	Range (%)	90-95	105-128	90-102	83-98	———	88-101
1:8	Average % of Expected	104	113	88	85	———	96
	Range (%)	98-110	98-125	78-95	73-99	———	91-100

SAMPLE VALUES

Serum/Plasma/Saliva/Urine - Samples from apparently healthy volunteers were evaluated in this assay. No medical histories were available for the donors used in this study.

Note: *Samples were diluted prior to assay as described in the Sample Preparation section.*

MMP-1

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=44)	5842	100	971-28,749
Heparin plasma (n=43)	1108	88	ND-2586
Platelet-poor heparin plasma (n=33)	2819	30	ND-18,229
Saliva (n=12)	2520	8	ND-2520
Urine (n=13)	———	0	———

MMP-2

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=44)	339,886	100	205,144-535,298
Heparin plasma (n=43)	350,972	100	240,720-498,340
Platelet-poor heparin plasma (n=33)	309,166	100	197,448-480,996
Saliva (n=12)	51,578	58	ND-104,076
Urine (n=13)	———	0	———

MMP-3

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum* (n=43)	11,428	100	2995-21,984
Heparin plasma* (n=42)	12,659	100	3826-38,693
Platelet-poor heparin plasma* (n=32)	11,639	100	4209-24,534
Saliva (n=12)	———	0	———
Urine (n=13)	———	0	———

MMP-7

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=44)	2405	100	1154-4575
Heparin plasma (n=43)	2379	100	1245-4211
Platelet-poor heparin plasma (n=33)	2073	100	70.0-3588
Saliva* (n=11)	16,691	100	896-42,636
Urine (n=13)	5672	100	144-13,408

ND=Non-detectable
*One sample assayed read above the top standard and was not included in this summary.

MMP-8

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=44)	15,908	100	2692-50,121
Heparin plasma (n=43)	4984	95	ND-25,611
Platelet-poor heparin plasma (n=33)	2396	30	ND-6709
Saliva* (n=11)	101,666	100	24,320-270,216
Urine (n=13)	26,606	38	ND-103,433

MMP-9

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=44)	434,868	100	154,950-976,240
Heparin plasma (n=43)	NR	NR	NR
Platelet-poor heparin plasma (n=33)	26,552	100	14,201-52,431
Saliva* (n=11)	243,226	100	63,540-744,336
Urine (n=13)	22,891	38	ND-71,215

NR=Not Recommended

*One sample assayed read above the top standard and was not included in this summary.

MMP-13

All serum, plasma, saliva, and urine samples tested for MMP-13 were non-detectable.

Cell culture supernates - USOS human osteocarcinoma cells were grown to 100% confluency in McCoy's 5a media supplemented with 15% fetal bovine serum and 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot of the cell culture supernate was removed and assayed in the Mosaic ELISA.

Cell Line	(pg/mL)						
	MMP-1	MMP-2	MMP-3	MMP-7	MMP-8	MMP-9	MMP-13
U2OS	ND	55,264	215	110	ND	2700	622

ND=Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant MMPs.

The following factors were assayed for cross-reactivity and interference in the Mosaic Human MMP Panel. Less than 1% cross-reactivity or interference was observed except where noted.

Recombinant human:
ADAM8
ADAM9
ADAM10
ADAM12
ADAM15
ADAM19
ADAMTS1
ADAMTS4
ADAMTS5
ADAMTS13
Lipocalin-1
Lipocalin-2/NGAL
MMP-10
MMP-12
TACE/ADAM17
TIMP-2
TIMP-4

Recombinant mouse:
ADAM9
ADAM10
ADAM15
ADAM19
Lipocalin-2/NGAL
MMP-3
MMP-7
MMP-8
MMP-9
MMP-12
TACE/ADAM17
TIMP-2

Recombinant rat:
Lipocalin-2/NGAL
MMP-8

Human TIMP-1 intereferes with MMP-9 at levels ≥ 3.13 ng/mL.

REFERENCES

1. Opdenakker, G. and J. Van Damme (1992) Cytokine **4**:251.
2. Rydlova, M. *et al.* (2008) Anticancer Res. **28**:1389.

Substrates 1 and 2 are comprised of TMA-6, a product of Lumigen, Inc., Southfield, Michigan, USA, and are covered by the following:

US Patent Numbers: 5,922,558 and 6,858,733

International Patent Numbers: 733,086, 1,019,525, 2,300,071, 1,015,461, 2,002,352,881, ZL02805225.0, and 1,456,716