Quantikine®

Human Pro-MMP-10 Immunoassay

Catalog Number DM1000

For the quantitative determination of human Pro-Matrix Metalloproteinase 10 (Pro-MMP-10) 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.

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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 (ECM). 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, α_2-macroglobulins and tissue inhibitors of metalloproteinases (TIMPs).

MMP-10 (also referred to as stromelysin-2 and transin-2) expression has been observed in several normal tissues (3). Expression of MMP-10 and MMP-3 (stromelysin-1 or transin-1) is differentially regulated. For example, MMP-10, not MMP-3, is strongly expressed in osteoclasts and most mononuclear cells within the bone marrow, and is induced in human keratinocytes by cytokines (4, 5). The latter has been observed during normal wound repair (6, 7). MMP-10 is also expressed in carcinoma cells of the human head, neck and lung carcinomas (8). MMP-10 overexpression in the diabetic corneal epithelium may be the major contributor to the observed changes in diabetic retinopathy (9).

MMP-10 is secreted from the cells as a pro-enzyme. The N-terminal pro domain contains the cysteine switch motif conserved in MMPs that maintains MMP-10 in the latent state (10). Activation of the proenzyme results in the removal of the pro domain. MMP-10 activation can be achieved *in vitro* by proteases such as itself and by chemicals such as 4-aminophenylmercuric acetate (11). The resulting mature and active enzyme consists of a catalytic domain with a zinc-binding motif conserved in metzincins (12, 13). A short hinge peptide links the catalytic domain to the C-terminal hemopexin-like domain. The active MMP-10 is capable of cleaving several proteins related to wound repair such as collagens III and IV, gelatin, nidogen, laminin-1, elastin and proteoglycans (6). The active enzyme also activates pro-MMP-1, -7, -8, and -9 (11). Therefore, MMP-10 may play an important role in the extracellular matrix turnover under various pathological conditions either by itself or in concert with other MMPs.

The Quantikine Human Pro-MMP-10 Immunoassay is a 4.5 hour solid phase immunoassay designed to measure Pro-MMP-10 in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant human Pro-MMP-10, and antibodies raised against the recombinant protein. This kit will not detect human active MMP-10 existing in either free or TIMP-bound form. Natural human Pro-MMP-10 showed dose-response curves that were parallel to the standard curves obtained using the recombinant Quantikine kit standards, indicating that the Quantikine kit can be used to determine relative levels of natural human Pro-MMP-10.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for Pro-MMP-10 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Pro-MMP-10 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for Pro-MMP-10 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Pro-MMP-10 bound in the initial step. The color development is stopped and the intensity of the color is measured.

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, 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.
- This assay is designed to eliminate interference by enzymes, proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

Pro-MMP-10 Microplate (Part 892144) - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against human MMP-10.

Pro-MMP-10 Conjugate (Part 892145) - 21 mL of monoclonal antibody against human Pro-MMP-10 conjugated to horseradish peroxidase with preservatives.

Pro-MMP-10 Standard (Part 892146) - 50 ng of recombinant human MMP-10 in a buffered protein base with preservatives; lyophilized.

Assay Diluent RD1-34 (Part 895265) - 11 mL of a buffered protein base with preservatives.

Calibrator Diluent RD5-10 (Part 895266) - 21 mL of a buffered protein base with preservatives.

Wash Buffer Concentrate (Part 895003) - 21 mL of a 25-fold concentrated solution of buffered surfactant with preservatives.

Color Reagent A (Part 895000) - 12.5 mL of stabilized hydrogen peroxide.

Color Reagent B (Part 895001) - 12.5 mL of stabilized chromogen (tetramethylbenzidine).

Stop Solution (Part 895032) - 6 mL of 2 N sulfuric acid.

Plate Covers - 4 adhesive strips.

STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 - 8° C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="9">Opened/ Reconstituted Reagents</td><td>Diluted Wash Buffer</td><td rowspan="8">May be stored for up to 1 month at 2 - 8° C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Assay Diluent RD1-34</td></tr>
<tr><td>Calibrator Diluent RD5-10</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>Unmixed Color Reagent A</td></tr>
<tr><td>Unmixed Color Reagent B</td></tr>
<tr><td>Standard</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.*</td></tr>
</table>

*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.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- 500 mL graduated cylinder.
- Human MMP-10 Controls (optional; available from R&D Systems).
- **Polypropylene test tubes** for dilution.

PRECAUTION

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

SAMPLE COLLECTION AND STORAGE

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 before centrifugation 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.

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

SAMPLE PREPARATION

Use polypropylene tubes.

Serum and plasma samples require a 2-fold dilution. A suggested 2-fold dilution is 150 μL of sample + 150 μL of Calibrator Diluent RD5-10.

Cell culture supernate samples may require dilution.

REAGENT PREPARATION

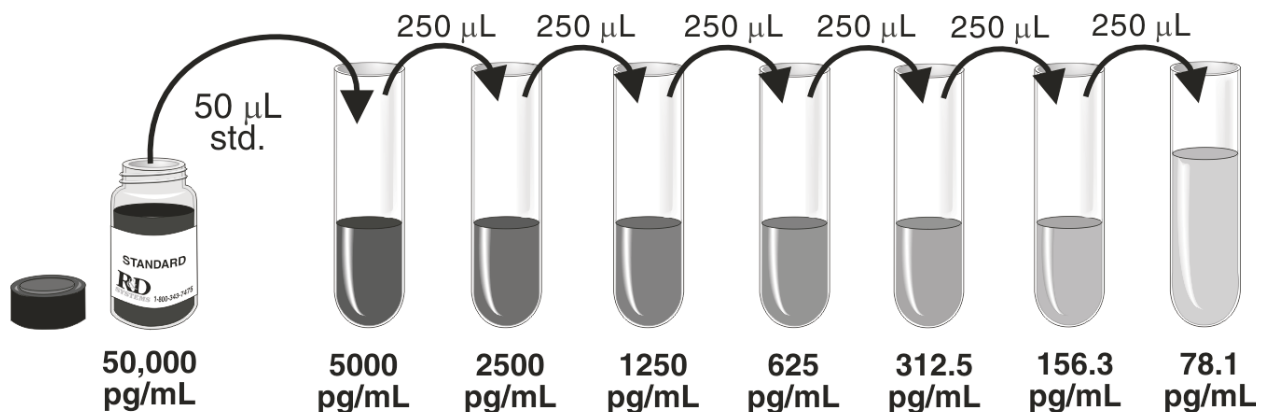
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into 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. 200 μL of the resultant mixture is required per well.

Pro-MMP-10 Standard - Reconstitute the Pro-MMP-10 Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a stock solution of 50,000 pg/mL. Allow the standard to sit for 30 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 450 μL of Calibrator Diluent RD5-10 into the 5000 pg/mL tube. Pipette 250 μL Calibrator Diluent RD5-10 into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 5000 pg/mL standard serves as the high standard. Calibrator Diluent RD5-10 serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

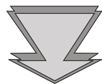
Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards, and controls be assayed in duplicate.

1. Prepare all reagents, working standards, 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 100 μL of Assay Diluent RD1-34 to each well.
4. Add 50 μL of Standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12” orbit) set at 500 ± 50 rpm. A plate layout is provided to record standards and samples assayed.
5. 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.
6. Add 200 μL of Pro-MMP-10 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 μL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50 μL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, 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.

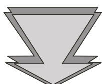
*Serum and plasma samples require dilution. See Sample Preparation section.

ASSAY PROCEDURE SUMMARY

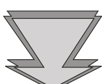
1. Prepare reagents, working standards, and samples as instructed.



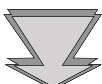
2. Add 100 µL Assay Diluent RD1-34 to each well.



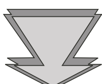
3. Add 50 µL Standard, control, or sample* to each well. Incubate 2 hours at RT on a horizontal orbital microplate shaker (0.12") set at 500 ± 50 rpm.



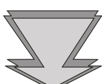
4. Aspirate and wash 4 times.



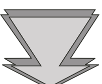
5. Add 200 µL Conjugate to each well. Incubate 2 hours at RT on the shaker.



6. Aspirate and wash 4 times.



7. Add 200 µL Substrate Solution to each well. Incubate 30 minutes at RT **on the benchtop. Protect from light.**



8. Add 50 µL Stop Solution to each well. Read at 450 nm within 30 minutes. λ correction 540 or 570 nm

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

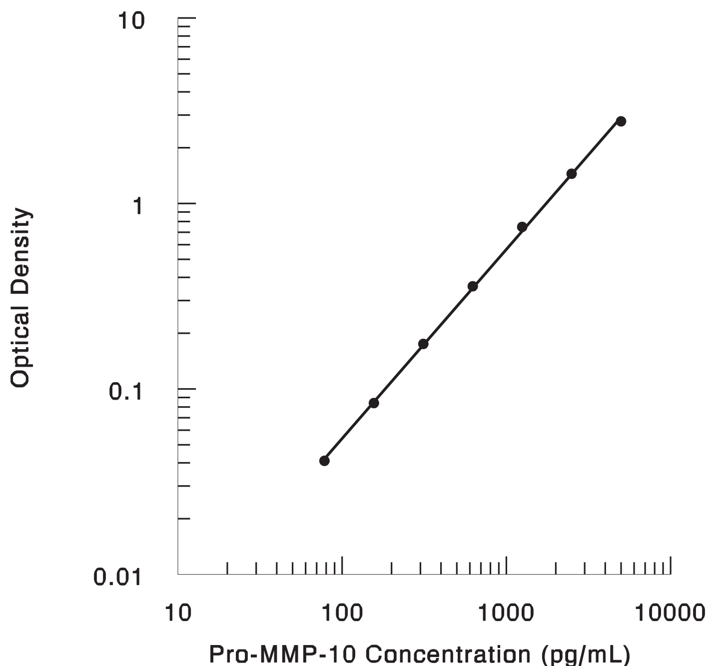
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

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 Pro-MMP-10 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	0.009 0.009	0.009	—
78.1	0.050 0.050	0.050	0.041
156.3	0.091 0.095	0.093	0.084
312.5	0.181 0.186	0.184	0.175
625	0.363 0.371	0.367	0.358
1250	0.731 0.782	0.757	0.748
2500	1.449 1.460	1.455	1.446
5000	2.766 2.796	2.781	2.772

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.
- 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 wash 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. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

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 separate assays to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (ng/mL)	515	2497	3442	515	2542	3592
Standard deviation	22.9	115	137	30.4	119	153
CV (%)	4.4	4.6	4.0	5.9	4.7	4.3

RECOVERY

The recovery of Pro-MMP-10 spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	99	88 - 109%

LINEARITY

To assess the linearity of the assay, samples containing or spiked with high concentrations of Pro-MMP-10 were serially diluted with Calibrator Diluent RD5-10 to produce samples with values within the dynamic range of the assay.

		Spiked cell culture media (n=4)	Cell culture supernates (n=5)	Serum* (n=5)	Heparin plasma* (n=5)
1:2	Average % of Expected	104	100	104	104
	Range (%)	102 - 106	97 - 103	99 - 111	102 - 105
1:4	Average % of Expected	103	102	106	106
	Range (%)	101 - 104	98 - 108	98 - 113	97 - 111
1:8	Average % of Expected	103	109	111	112
	Range (%)	100 - 105	104 - 113	—	—
1:16	Average % of Expected	99	104		
	Range (%)	94 - 102	—	—	—

*Samples were diluted 2-fold prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Thirty-one assays were evaluated and the minimum detectable dose (MDD) of Pro-MMP-10 ranged from 0.08 - 15.1 pg/mL. The mean MDD was 4.13 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 human MMP-10 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples drawn from apparently healthy volunteers were evaluated for the presence of Pro-MMP-10 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=60)	770	305 - 1727	356
Heparin plasma (n=35)	826	402 - 1799	323

Cell Culture Supernates -
Human peripheral blood mononuclear cells (5 x 10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal calf serum, 50 μM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 10 μg/mL PHA for 5 days. Aliquots of the cell culture supernate were removed and assayed for levels of natural Pro-MMP-10. Unstimulated and stimulated samples measured 560 pg/mL and 660 pg/mL, respectively.

Human osteogenic sarcoma cells (U2-OS) were grown to 100% confluency in McCoy's 5a media with 15% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate. An aliquot was removed, assayed for detectable levels of Pro-MMP-10 and measured 203 pg/mL.

Human osteosarcoma cells (MG-63) were cultured in DMEM with 10% fetal bovine serum and grown to 100% confluence. An aliquot was removed, assayed for detectable levels of Pro-MMP-10 and measured 190 pg/mL.

Human fibroblast cells (HT 1080) were cultured in DMEM with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate. Cells were stimulated with PMA. An aliquot was removed, assayed for detectable levels of Pro-MMP-10, and measured 36,250 pg/mL.

SPECIFICITY

This assay recognizes recombinant and natural human Pro-MMP-10. The factors listed below were prepared at 200 ng/mL in Calibrator Diluent RD5-10 and assayed for cross-reactivity. Preparations of the following factors at 200 ng/mL in a mid-range recombinant human MMP-10 control were assayed for interference. No significant cross-reactivity or inteference was observed.

Recombinant human:	**Recombinant mouse:**
MMP-1	MMP-3
MMP-2	MMP-9
MMP-3	
MMP-7	
MMP-8	
MMP-9	
MMP-13	
TIMP-1	
TIMP-2	
TIMP-3	
TIMP-4	

REFERENCES

1. Nagase, H. and J.F. Woessner, Jr. (1999) J. Biol. Chem. **274**:2191.
2. Parks, W.C. and R.P. Mecham (1998) *Matrix Metalloproteinases*, Academic Press, San Diego.
3. Matrisian, L.M. (1998) in *Handbook of Proteolytic Enzymes*, Barrett, A.J. *et al.* eds., Academic Press, San Diego, pp. 1178 - 1180.
4. Windsor, L.J. *et al*. (1993) J. Biol. Chem. **268**:17341.
5. Bord, S. *et al.* (1998) Bone **23**:7.
6. Rechardt, O. *et al*. (2000) J. Invest. Dermatol. **115**:778.
7. Madlener, M. *et al*. (1996) Biochem. J. **320**:659.
8. Muller, D. *et al.* (1991) Int. J. Cancer **48**:550.
9. Saghizadeh, M. *et al.* (2001) Am. J. Pathol. **158**:723.
10. Van Wart, H.E. and H. Birkedal-Hansen (1990) Proc. Natl. Acad. Sci. USA **87**:5578.
11. Nakamura, H. *et al*. (1998) Eur. J. Biochem. **253**:67.
12. Jiang, W. and J.S. Bond (1992) FEBS Lett. **312**:110.
13. Bode, W. *et al.* (1993) FEBS Lett. **331**:134.

PLATE LAYOUT

Use this plate layout as a record of standards and samples assayed.

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