Quantikine® ELISA

Total MMP-2 Immunoassay

Catalog Numbers MMP200
SMMP200
PMMP200

For the quantitative determination of human, mouse, rat, porcine, or canine active, pro-, and TIMP complexed Matrix Metalloproteinase 2 (Total MMP-2) 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 the extracellular matrix and in the processing of a variety of biological molecules (1). They play important roles in many normal physiological processes such as embryonic development, morphogenesis, and tissue remodeling (1, 2). MMPs also participate in many pathological processes including inflammation, cancer, and cardiovascular disease (3-5). MMP-2 (Gelatinase A) is widely expressed during development and is upregulated at sites of tissue damage, inflammation, and in stromal cells surrounding the invading front of metastatic tumors (2, 5, 6). It promotes the progression of tumors to an angiogenic and metastatic phenotype *in vivo* (7, 8). MMP-2 is elevated in many body fluids in these environments (9-12).

Human MMP-2 is secreted as a nonglycosylated 72 kDa proenzyme (pro-MMP-2) that contains an 80 amino acid (aa) propeptide and a 551 aa mature region (13, 14). The propeptide contains a cysteine switch motif that is conserved in MMPs and maintains MMP-2 in a latent state (14, 15). Removal of the propeptide can be catalyzed by membrane-type MMPs (MT-MMPs) or by the serine proteases Thrombin and activated Protein C (16-19). The resulting mature and active enzyme consists of a catalytic domain which is interrupted by three contiguous fibronectin type II-like domains and a C-terminal hemopexin-like domain (13, 15). Both the pro- and active forms of human MMP-2 share 96-97% aa sequence identity with mouse, rat, porcine, and canine pro-MMP-2.

While the amounts of newly synthesized MMPs are regulated mainly at the level of transcription, the proteolytic activities of existing MMPs are controlled through the activation of proenzymes and the inhibition of active enzymes by endogenous inhibitors such as α_2-Macroglobulin and Tissue Inhibitors of Metalloproteinases (TIMPs) (20, 21). TIMPs 1-4 inhibit active MMP-2 through tight but non-covalent binding of their N-terminal domains to the catalytic domain of MMP-2 in a 1:1 stoichiometry (1, 5, 21). In addition, TIMP-2 and -3 can tether pro-MMP-2 into cell surface ternary complexes with MT-MMPs (16, 17).

Together with MMP-9 (Gelatinase B), MMP-2 degrades Gelatin (denatured Collagen) and type IV Collagen, the major component of basement membranes (13). It can also degrade the matrix proteins Collagens V, VII and X, Decorin, Elastin, and Fibronectin (5, 13). MMP-2 processes and modulates the functions of many other vasoactive and proinflammatory molecules including Adrenomedullin, Big Endothelin-1, Calcitonin gene-related peptide, CCL7/MCP-3, CXCL12/SDF-1, Galectin-3, IGFBP-3, IL-1β, S100A8, and S100A9 (1, 3, 5, 7).

The Quantikine Total MMP-2 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure MMP-2 in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant mouse/rat pro-MMP-2 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural MMP-2 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 mass values for naturally occurring MMP-2.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for Total MMP-2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any MMP-2 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for Total MMP-2 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 Total MMP-2 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, 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.
- 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.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	CATALOG # MMP200	CATALOG # SMMP200	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Total MMP-2 Microplate	894404	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for Total MMP-2.	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.*
Total MMP-2 Conjugate	894405	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for Total MMP-2 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Total MMP-2 Standard	894406	1 vial	6 vials	Recombinant mouse/rat pro-MMP-2 in a buffered protein base with preservatives; lyophilized. *Refer to vial label for reconstitution volume.*	
Assay Diluent RD1-116	895858	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5P Concentrate	895151	1 vial	6 vials	21 mL/vial of a concentrated buffered protein base with preservatives. *Used diluted 1:5 in this assay.*	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

MMP200 contains sufficient materials to run an ELISA on one 96 well plate.
SMMP200 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PMMP200). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

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.
- 100 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.
- MMP-2 Controls (optional; available from R&D Systems).

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.

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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.

Human Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature 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.

Mouse 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.

Rat/Porcine/Canine Serum - Allow blood samples to clot for 2 hours at room temperature before centrifugation for 30 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.*
Grossly hemolyzed samples are not suitable for use in this assay.

SAMPLE PREPARATION

Human/canine serum and plasma samples require a 20-fold dilution. A suggested 20-fold dilution is 20 µL of sample + 380 µL of Calibrator Diluent RD5P (diluted 1:5).*

Porcine serum and plasma samples require a 40-fold dilution. A suggested 40-fold dilution is 10 µL of sample + 390 µL of Calibrator Diluent RD5P (diluted 1:5).

Mouse/rat serum and plasma samples require a 100-fold dilution. A suggested 100-fold dilution is 10 µL of sample + 990 µL of Calibrator Diluent RD5P (diluted 1:5).

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

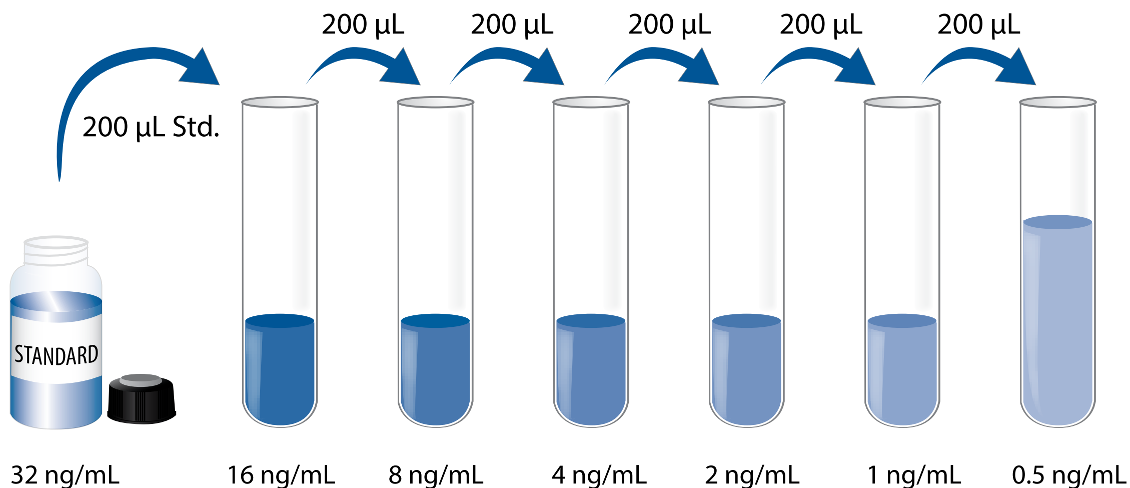
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

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.

Calibrator Diluent RD5P (diluted 1:5) - Add 20 mL of Calibrator Diluent RD5P Concentrate to 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent RD5P (diluted 1:5).

Total MMP-2 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Total MMP-2 Standard with Calibrator Diluent RDP (diluted 1:5). This reconstitution produces a stock solution of 32 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD5P (diluted 1:5) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 32 ng/mL standard serves as the high standard. Calibrator Diluent RD5P (diluted 1:5) serves as the zero standard (0 ng/mL).



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ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards 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 50 µL of Assay Diluent RD1-116 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 Total MMP-2 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.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

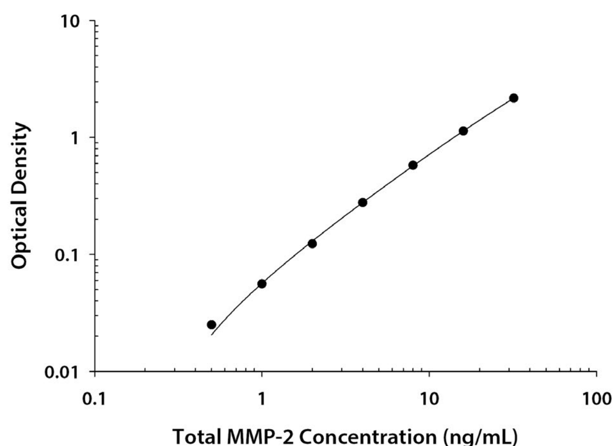
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 Total MMP-2 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.



(ng/mL)	O.D.	Average	Corrected
0	0.009 0.009	0.009	—
0.5	0.033 0.034	0.034	0.025
1	0.065 0.065	0.065	0.056
2	0.130 0.134	0.132	0.123
4	0.283 0.288	0.286	0.277
8	0.584 0.587	0.586	0.577
16	1.140 1.141	1.141	1.132
32	2.150 2.191	2.171	2.162

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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 technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (ng/mL)	4.30	11.2	22.8	4.26	11.1	21.5
Standard deviation	0.302	0.420	0.828	0.275	0.738	1.51
CV (%)	7.0	3.8	3.6	6.5	6.6	7.0

RECOVERY

The recovery of Total MMP-2 spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	99	90-105%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of Total MMP-2 were diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

Human Samples		Cell culture media (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	106	97	93	95
	Range (%)	99-113	90-103	87-98	90-100
1:4	Average % of Expected	104	98	99	95
	Range (%)	98-113	92-108	91-111	88-100
1:8	Average % of Expected	105	93	97	92
	Range (%)	95-117	89-98	87-107	85-99
1:16	Average % of Expected	99	88	92	85
	Range (%)	93-108	80-98	83-107	80-88

Note: *Mouse, rat, porcine, and canine samples were evaluated and no significant difference in linearity was observed from the data above.*
*Samples were diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of MMP-2 ranged from 0.014-0.082 ng/mL. The mean MDD was 0.033 ng/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/rat pro-MMP-2 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of Total MMP-2 in this assay. No medical histories were available for the donors used in this study.

Human Samples	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=35)	241	139-356	46.9
EDTA plasma (n=35)	201	141-278	37.8
Heparin plasma (n=35)	218	155-342	43.9

Mouse Samples	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=10)	1028	854-1202	117
EDTA plasma (n=5)	704	556-872	140
Heparin plasma (n=5)	1044	712-1166	189

Rat Samples	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=4)	1146	548-1398	404
EDTA plasma (n=4)	908	610-1338	325
Heparin plasma (n=4)	1709	1126-2796	742

Porcine Samples	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=4)	308	220-408	85.4
EDTA plasma (n=4)	268	186-372	78.1
Heparin plasma (n=4)	294	242-346	50.3

Canine Samples	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=4)	32.8	24.4-45.4	9.0
EDTA plasma (n=4)	38.1	29.8-46.8	7.2
Heparin plasma (n=4)	31.0	23.8-40.4	7.0

SAMPLE VALUES *CONTINUED*

Cell Culture Supernates:

MG-63 human osteosarcoma cells were were cultured in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were grown for 4 days at 37 °C. An aliquot of the cell culture supernate was removed, assayed for levels of natural Total MMP-2, and measured 152 ng/mL.

3T3-L1 mouse embryonic fibroblast adipose-like cells (2 x 10^6 cells/flask) were cultured in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 3 days. Cells were transferred to 50 mL fresh media and cultured for an additional 4 days. An aliquot of the culture supernate was removed, assayed for natural Total MMP-2, and measured 248 ng/mL.

SPECIFICITY

This assay recognizes recombinant MMP-2 and natural human, mouse, rat, porcine, and canine active, pro-, and TIMP complexed MMP-2.

The factors listed below were prepared at 200 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 200 ng/mL in a mid-range recombinant MMP2 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Hemopexin
MMP-1
MMP-10
MMP-12 (Hemopexin domain)
MMP-12 (pro)
MMP-13
MMP-14
MMP-16 (pro)
TIMP-1
TIMP-2
TIMP-3
TIMP-4

Recombinant mouse:

ADAM-9
ADAM-10
ADAM-19
ADAM-33
ADAMTS1
Lipocalin-2
MMP-3
MMP-7
MMP-8
MMP-9
MMP-12
TACE
TIMP-1
TIMP-4

Recombinant rat:

TIMP-1

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

NOTES

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