Quantikine® ELISA

Human MMP-2 Immunoassay

Catalog Number DMP2F0
SMP2F0
PDMP2F0

For the quantitative determination of Matrix Metalloproteinase 2 (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 an important role 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 conditions (9-12).

Human MMP-2 is secreted as a non-glycosylated 72 kDa pro-enzyme (pro-MMP-2) that contains an 80 amino acid (aa) pro domain and a 551 aa mature region (13, 14). The pro domain contains a cysteine switch motif that is conserved in MMPs and maintains MMP-2 in a latent state (14, 15). Removal of the pro domain can be initiated 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). Human pro-MMP-2 shares 97% aa sequence identity with mouse and rat 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 pro-enzymes and the inhibition of active enzymes by endogenous inhibitors such as α2-macroglobulin and tissue inhibitors of metalloproteinases (TIMPs) (20, 21). TIMP-1 through TIMP-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 collagens V, VII, and X, decorin, elastin, and fibronectin (5, 13). MMP-2 processes and modulates the functions of many other vasoactive and pro-inflammatory 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 Human 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 CHO cell-expressed recombinant human MMP-2 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human MMP-2 showed dose response 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 human MMP-2.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for 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 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 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 enzymes, binding 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.

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 # DMP2F0	CATALOG # SMP2F0	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
MMP-2 Microplate	893692	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against MMP-2.	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.*
MMP-2 Conjugate	893693	1 vial	6 vials	21 mL/vial of a polyclonal antibody against MMP-2 conjugated to horseradish peroxidase with preservatives.	May be stored for up to1 month at 2-8 °C.*
MMP-2 Standard	893694	1 vial	6 vials	100 ng/vial of recombinant human MMP-2 in a buffered protein solution with preservatives; lyophilized.	
Assay Diluent RD1-74	895809	1 vial	6 vials	11 mL/vial of a buffered protein solution with preservatives.	
Calibrator Diluent RD5-32	895810	1 vial	6 vials	21 mL/vial of a buffered protein solution with preservatives.	
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.

DMP2F0 contains sufficient materials to run an ELISA on one 96 well plate.
SMP2F0 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PDMP2F0). 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.
- 500 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- **Polypropylene** test tubes for dilution of standards and samples.
- Human 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.

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 - 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.

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: *EDTA plasma is not suitable for use in this assay .*
Citrate plasma has not been validated for use in this assay.

SAMPLE PREPARATION

Serum and plasma samples require a 10-fold dilution. A suggested 10-fold dilution is 20 µL of sample + 180 µL of Calibrator Diluent RD5-32.

REAGENT PREPARATION

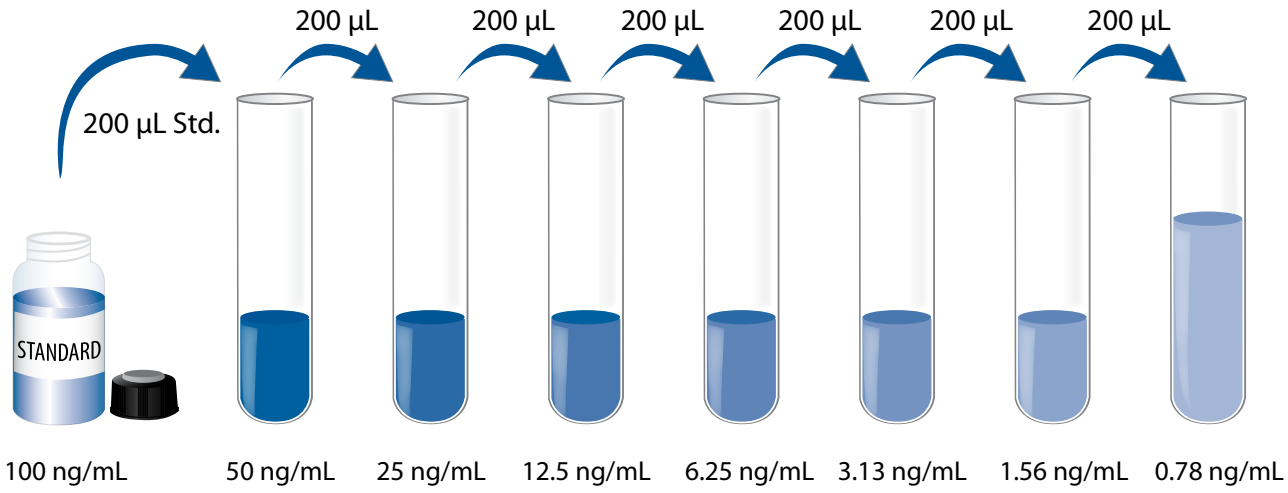
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. To prepare enough Wash Buffer for one plate, 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.

MMP-2 Standard - Reconstitute the MMP-2 Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a stock solution of 100 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.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 50 ng/mL standard serves as the high standard. The Calibrator Diluent serves as the zero standard (0 ng/mL).



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 100 µL of Assay Diluent RD1-74 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.
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 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.

*Serum/plasma samples require dilution. See the Sample Preparation section.

CALCULATION OF RESULTS

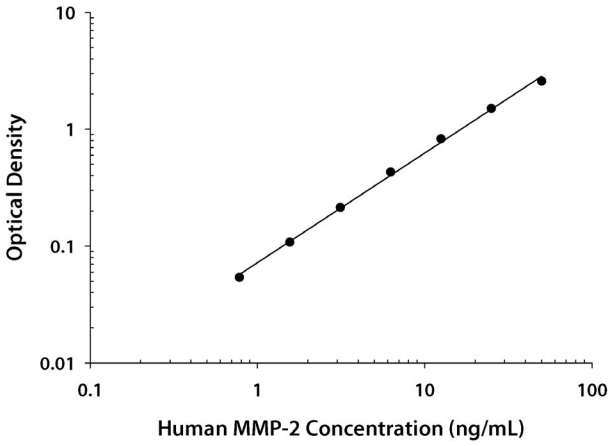
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a log/log 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 a log/log graph. The data may be linearized by plotting the log of the MMP-2 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If the 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	0.023 0.025	0.024	—
0.78	0.078 0.078	0.078	0.054
1.56	0.131 0.133	0.132	0.108
3.13	0.237 0.238	0.238	0.214
6.25	0.451 0.457	0.454	0.430
12.5	0.842 0.860	0.851	0.827
25	1.512 1.547	1.530	1.506
50	2.592 2.607	2.600	2.576

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)	3.30	12.1	18.9	3.96	12.4	19.6
Standard deviation	0.18	0.65	1.1	0.39	0.86	1.1
CV (%)	5.5	5.4	5.8	9.8	6.9	5.6

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	106	91-120%
Serum* (n=4)	84	78-89%
Heparin plasma* (n=4)	90	73-105%

*Samples were diluted prior to assay.

LINEARITY

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

		Cell culture supernates (n=2)	Serum* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	103	105	105
	Range (%)	93-112	101-111	102-108
1:4	Average % of Expected	101	106	104
	Range (%)	94-108	101-112	102-107
1:8	Average % of Expected	98	104	101
	Range (%)	94-103	99-111	99-103
1:16	Average % of Expected	93	101	96
	Range (%)	92-93	97-110	94-97

*Samples were diluted prior to assay.

SENSITIVITY

Fifty-one assays were evaluated and the minimum detectable dose (MDD) of MMP-2 ranged from 0.016-0.289 ng/mL. The mean MDD was 0.047 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 CHO cell-expressed recombinant human MMP-2 produced at R&D Systems.

SAMPLE VALUES

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

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=35)	199	161-301	24.6
Heparin plasma (n=35)	189	155-323	29.8

Cell Culture Supernates:
Human peripheral blood leukocytes (1 x 10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal calf serum, 5 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated for 1 and 5 days with 10 µg/mL PHA. Aliquots of the cell culture supernates were removed and assayed for levels of natural MMP-2. No detectable levels were observed.

USOS human osteosarcoma cells were cultured in McCoy's 5a media and 15% fetal calf serum for five days. The cells were then cultured in McCoy's 5a media for one day. An aliquot of the cell culture supernate was removed, assayed for levels of natural MMP-2, and measured 16.5 ng/mL.

MG63 human osteosarcoma cells were thawed from MEM (NEAA) and 10% fetal bovine serum, rinsed in 10% trypsin, and then grown overnight at 37 °C. An aliquot of the cell culture supernate was removed, assayed for levels of natural MMP-2, and measured 87.0 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant (pro- and mature) human MMP-2.

The factors listed below were prepared at 500 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 500 ng/mL in a mid-range recombinant human MMP-2 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

ADAM-8
ADAM-9
ADAM-10
ADAM-12
ADAM-19
ADAM TS1
ADAM TS4
ADAM TS5
ADAM TS13
Lipocalin-1
Lipocalin-2
MMP-1
MMP-7
MMP-8
MMP-10
MMP-12
MMP-13
TIMP-1
TIMP-4

Recombinant mouse:

ADAM-9
ADAM-10
ADAM-19
Lipocalin-2
MMP-3
MMP-7
MMP-9
MMP-12
TACE
TIMP-1
TIMP-2

Recombinant rat:

Lipocalin-2
MMP-8
TIMP-1

Recombinant human TIMP-2 interferes at concentrations greater than 0.8 ng/mL. Therefore, this kit measures free MMP-2 but not total MMP-2.

This kit exhibits approximately 80% cross-reactivity with recombinant mouse MMP-2.

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