Quantikine® ELISA

Human Total MMP-3 Immunoassay

Catalog Number DMP300
SMP300
PDMP300

For the quantitative determination of human active and Pro-Matrix Metalloproteinase 3 (total MMP-3) 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-macroglobulins and tissue inhibitors of metalloproteinases (TIMPs).

MMP-3 (also referred to as stromelysin-1) may be expressed in fibroblasts, chondrocytes, endothelial cells, macrophages, vascular smooth muscle cells, osteoblasts, and keratinocytes in response to appropriate stimuli (3). Various agents regulate its biosynthesis. Inflammatory cytokines such as IL-1 and TNF-α, epidermal growth factor, platelet-derived growth factor, phorbol and oncogenic cellular transformation are the inductive agents. In comparison, retinoic acid, glucocorticoids, estrogen, progesterone and TGF-β suppress MMP-3 synthesis.

MMP-3 is secreted from the cells as a proenzyme. The proenzyme has been shown to stimulate plasminogen activation (4). The N-terminal pro-domain contains the cysteine switch motif conserved in MMPs that maintains MMP-3 in the latent state (5). Activation of the proenzyme results in the removal of the pro-domain. MMP-3 activation can be achieved *in vitro* by proteases such as itself, chyrotrypsin, neutrophil elastase and plasma kallikrein, and by mercury compounds (3). The resulting active enzyme consists of a catalytic domain with a zinc-binding motif conserved in metzincins (6,7). A short hinge peptide links the catalytic domain to the C-terminal hemopexin-like domain. The active MMP-3 is capable of cleaving types III, IV, IX and X collagen, aggrecan, fibronectin, laminin, IGFBP-3, serpins, and IL-1β. The active enzyme also activates proMMP-1, -8, -9, and -13. Therefore, it is suggested that MMP-3 may participate in physiological matrix turnover and pathological destruction of the tissue. For example, MMP-3 is required for the generation of a macrophage chemoattractant in a model of herniated disc resorption (8).

The Quantikine Human Total MMP-3 Immunoassay is a 4.5 hour solid phase immunoassay designed to measure total MMP-3 (pro- and active MMP-3) in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant human pro-MMP-3 and antibodies raised against the recombinant factor. Both antibodies also recognize recombinant human active MMP-3. Natural human MMP-3 showed dose-response curves that were parallel to the standard curves obtained using the recombinant Quantikine kit standards. These results indicate that this kit can be used to determine relative levels of natural human MMP-3.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for human MMP-3 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any MMP-3 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human MMP-3 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-3 (pro- and/or active) 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 variations 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 # DMP300	CATALOG # SMP300	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human MMP-3 (total) Microplate	890944	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for human MMP-3.	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.*
Human Total MMP-3 Standard	890946	1 vial	6 vials	Recombinant human pro-MMP-3 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store at ≤ -20 °C in a manual defrost freezer for up to 1 month.* Avoid repeated freeze-thaw cycles.
Human MMP-3 Conjugate	890945	1 vial	6 vials	21 mL/vial of polyclonal antibody specific for human MMP-3 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-52	895343	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives. *May contain a precipitate. Mix well before and during use.*	
Calibrator Diluent RD5-10	895266	2 vials	12 vials	21 mL/vial of a buffered protein base 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.

DMP300 contains sufficient materials to run an ELISA on one 96 well plate.
SMP300 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PDMP300). 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.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- 500 mL graduated cylinder.
- **Polypropylene** test tubes for dilution of standards and samples.
- Human Total MMP-3 Controls (optional; R&D Systems, Catalog # QC127).

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 and Citrate are not recommended as anticoagulants for use in this assay due to their chelating properties.*

SAMPLE PREPARATION

Use polypropylene tubes.

Cell culture supernate samples may require a 2-fold dilution. A suggested 2-fold dilution is 150 µL of sample + 150 µL of Calibrator Diluent RD5-10.

Serum and plasma samples require a 10-fold dilution. A suggested 10-fold dilution is 50 µL of sample + 450 µL of Calibrator Diluent RD5-10.

REAGENT PREPARATION

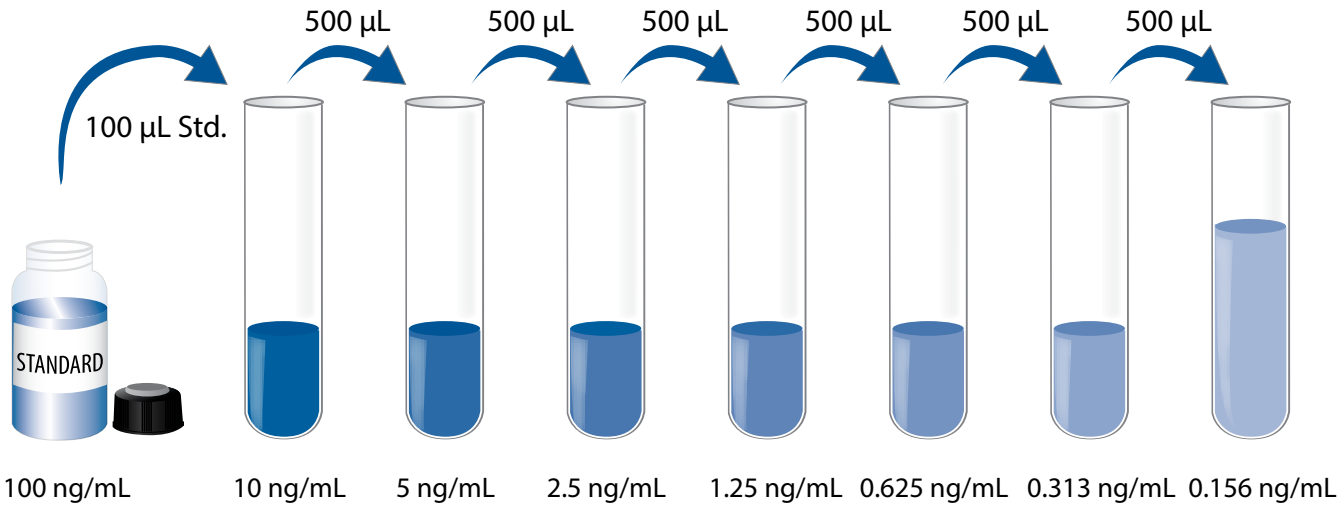
Bring all reagents to room temperature before use.

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.

Human Total MMP-3 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Total MMP-3 Standard with deionized or distilled water. This reconstitution produces a stock solution of 100 ng/mL. Allow the standard to sit for 30 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent RD5-10 into the 10 ng/mL tube. Pipette 500 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 10 ng/mL standard serves as the high standard. Calibrator Diluent RD5-10 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 standards, samples, 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 RD1-52 to each well. *Assay Diluent RD1-52 may contain a precipitate. Mix well before and during use.*
4. Add 100 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 Human MMP-3 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

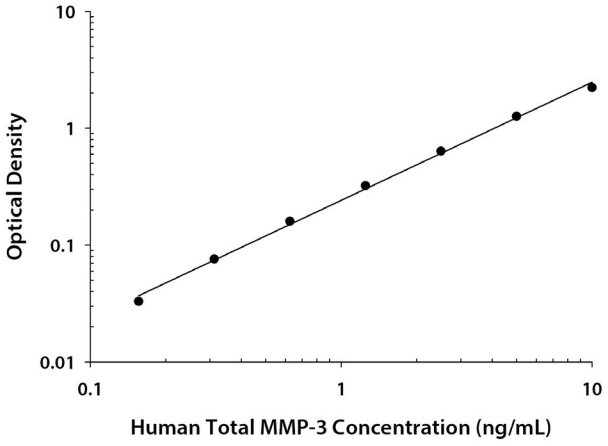
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a 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 human MMP-3 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

Since 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	0.014 0.014	0.014	—
0.156	0.046 0.048	0.047	0.033
0.313	0.087 0.092	0.090	0.076
0.625	0.171 0.176	0.174	0.160
1.25	0.330 0.341	0.336	0.322
2.5	0.657 0.644	0.650	0.636
5	1.263 1.289	1.276	1.262
10	2.211 2.264	2.238	2.224

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. 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	40	40	40
Mean (ng/mL)	0.962	3.21	5.87	0.994	3.08	5.70
Standard deviation	0.062	0.197	0.332	0.085	0.217	0.449
CV (%)	6.4	6.1	5.7	8.6	7.0	7.9

RECOVERY

The recovery of human MMP-3 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	96	87-104%
Serum* (n=5)	90	86-96%
Heparin plasma* (n=5)	92	85-106%

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

SENSITIVITY

Forty assays were evaluated and the minimum detectable dose (MDD) of human MMP-3 ranged from 0.002-0.045 ng/mL. The mean MDD was 0.009 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.

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of human MMP-3 were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture samples* (n=4)	Serum* (n=5)	Heparin plasma* (n=5)
1:2	Average % of Expected	105	99	97
	Range (%)	101-111	96-101	91-103
1:4	Average % of Expected	106	98	95
	Range (%)	97-112	91-108	89-105
1:8	Average % of Expected	104	91	91
	Range (%)	94-115	85-99	85-95
1:16	Average % of Expected	96	———	———
	Range (%)	88-108	———	———

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

CALIBRATION

This immunoassay is calibrated against highly purified NS0-expressed recombinant human pro-MMP-3 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human MMP-3 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)
Serum (n=60)	18.1	2.10-64.4
Heparin plasma (n=35)	14.5	1.88-45.9

Cell Culture Supernates:

U2OS human osteosarcoma cells were grown to 100% confluency in McCoy's 5A media supplemented with15% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot of the cell culture supernate was removed, assayed for human MMP-3, and measured 3.84 ng/mL.

MG-63 human osteosarcoma cells were grown to 100% confluency in DMEM supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot of the cell culture supernate was removed, assayed for human MMP-3, and measured 10.6 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human total MMP-3.

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 human MMP-3 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

MMP-1
MMP-2
MMP-7
MMP-8
MMP-9
MMP-13
TIMP-1
TIMP-2

Recombinant mouse:

MMP-9

Cross-reactivity was observed with recombinant human MMP-10 at concentrations > 4 ng/mL.

rhMMP-10 Concentration (ng/mL)	% Cross-reactivity
200	0.69
100	0.54
40	0.37
4	0.21

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