Quantikine® ELISA

Mouse Total MMP-3 Immunoassay

Catalog Number MMP300

For the quantitative determination of mouse Matrix Metalloproteinase 3 (MMP-3) concentrations in cell culture supernates and serum.

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-macroglobulin and tissue inhibitors of metalloproteinases (TIMPs).

Mouse 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. In addition, MMP-3 expression is highly regulated in various tumors and carcinoma cells, such as mouse skin tumors and mammary carcinoma cells (4, 5).

MMP-3 is secreted from the cells as a proenzyme. The proenzyme has been shown to stimulate plasminogen activation (6). The N-terminal pro-domain contains the cysteine switch motif conserved in MMPs that maintains MMP-3 in the latent state (7). 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, chymotrypsin, 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 (8, 9). 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 (10) and for initiation of the response in contact hypersensitivity (11).

The Quantikine® Mouse Total MMP-3 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure total mouse MMP-3 (pro-, active, and TIMP-complexed MMP-3) in cell culture supernates and mouse serum. It contains NS0-expressed recombinant mouse MMP-3 and antibodies raised against the recombinant factor. Natural mouse 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 mouse MMP-3.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse MMP-3 has been pre-coated onto a microplate. Standards, control 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 monoclonal antibody specific for mouse 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 the color develops in proportion to the amount of MMP-3 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 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.
- 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.

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
Mouse Total MMP-3 Microplate	892237	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse 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.*
Mouse Total MMP-3 Conjugate	892238	11 mL of a monoclonal antibody specific for mouse MMP-3 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Mouse Total MMP-3 Standard	892239	Recombinant mouse MMP-3 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Mouse MMP-3 Control	892240	Recombinant mouse MMP-3 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Assay Diluent RD1-79	895845	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-34	895828	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:10 in this assay.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* 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.
- 50 mL and 500 mL graduated cylinders.
- **Polypropylene** test tubes for dilution of standards and samples.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative 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. Refer to the SDS 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. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

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.

SAMPLE PREPARATION

Use polypropylene tubes.

Serum samples require a 20-fold dilution. A suggested 20-fold dilution is 10 µL of sample + 190 µL of Calibrator Diluent RD5-34 (diluted 1:10)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse MMP-3 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

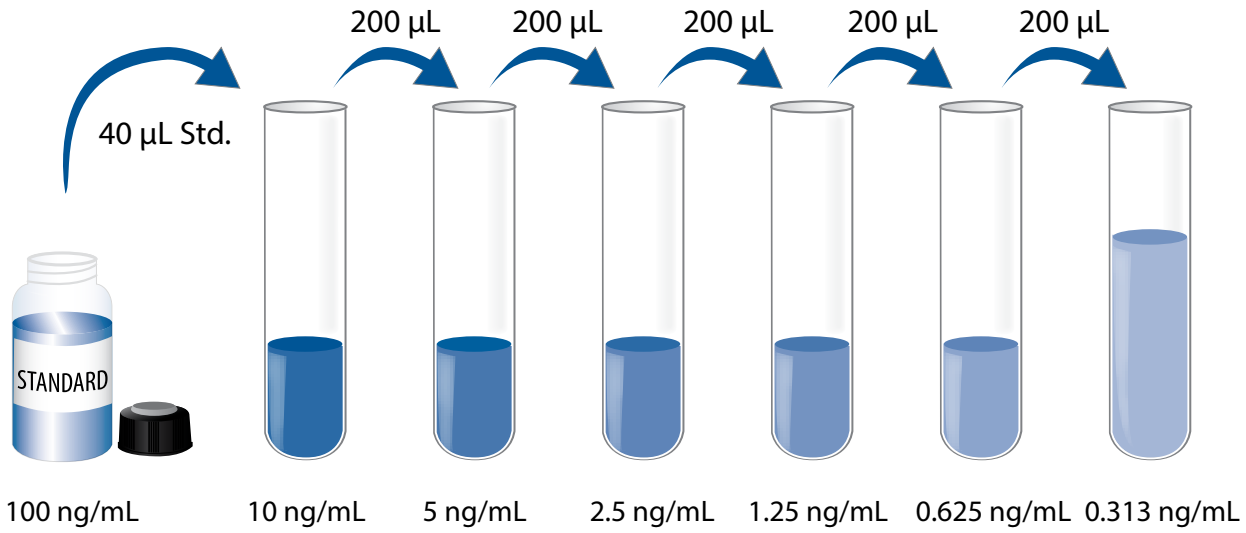
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Calibrator Diluent RD5-34 (diluted 1:10) - Add 5.0 mL of Calibrator Diluent RD5-34 to 45 mL of deionized or distilled water to prepare 50 mL of Calibrator Diluent RD5-34 (diluted 1:10).

Mouse Total MMP-3 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse Total MMP-3 Standard with 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 360 µL of Calibrator Diluent RD5-34 (diluted 1:10) into the 10 ng/mL tube. Pipette 200 µL 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 RD5-34 (diluted 1:10) 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, control, and samples be assayed in duplicate.

1. Prepare all reagents, working standards, control, 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 RD1-79 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.
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 100 µL of Mouse Total MMP-3 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 50 µL of Stop Solution to each well. 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

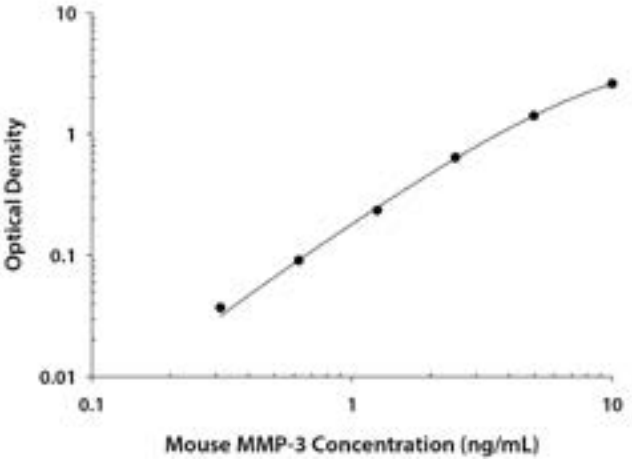
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 mouse MMP-3 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	0.023 0.024	0.024	—
0.313	0.059 0.062	0.061	0.037
0.625	0.114 0.116	0.115	0.091
1.25	0.259 0.259	0.259	0.235
2.5	0.656 0.676	0.666	0.642
5	1.415 1.462	1.439	1.415
10	2.632 2.640	2.636	2.612

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)	0.76	3.17	4.72	0.82	3.29	4.99
Standard deviation	0.04	0.05	0.09	0.08	0.19	0.37
CV (%)	5.3	1.6	1.9	9.8	5.8	7.4

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	99	92-106%
Serum* (n=4)	98	93-107%

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

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of mouse MMP-3 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum* (n=4)
1:2	Average % of Expected	106	99
	Range (%)	103-107	92-105
1:4	Average % of Expected	109	98
	Range (%)	103-112	89-105
1:8	Average % of Expected	110	97
	Range (%)	103-115	90-104
1:16	Average % of Expected	110	102
	Range (%)	109-115	90-109

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

SENSITIVITY

Fifty-four assays were evaluated and the minimum detectable dose (MDD) of mouse MMP-3 ranged from 0.005-0.053 ng/mL. The mean MDD was 0.019 ng/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

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

SAMPLE VALUES

Serum - Samples were evaluated for detectable levels of mouse MMP-3 in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=35)	49.6	13.7-154	32.1

Cell Culture Supernates - ST-2 mouse bone marrow-derived stromal cells were grown in RPMI with 10% fetal bovine serum, 2 mM L-glutamine, 1000 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were grown to confluence and fed every 3-4 days for 4 weeks. An aliquot of the cell culture supernate was removed and assayed for mouse MMP-3. No detectable levels were observed.

SPECIFICITY

This assay recognizes natural and recombinant mouse MMP-3 (pro-, active, and TIMP-complexed).

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

Recombinant mouse:

MMP-2
MMP-9
TIMP-1

Recombinant rat:

TIMP-1

Recombinant human:

MMP-1
MMP-2
MMP-3
MMP-7
MMP-8
MMP-9
MMP-10
MMP-12 (Catalytic Domain)
MMP-13
TIMP-1
TIMP-2
TIMP-3
TIMP-4

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