# Quantikine® ELISA

## Mouse Total MMP-9 Immunoassay

Catalog Number MMPT90

For the quantitative determination of mouse Matrix Metalloproteinase 9 (MMP-9) concentrations in cell culture supernates, serum, and platelet-poor 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, 2). They also participate in many pathological processes such as arthritis, cancer, and cardiovascular disease (3). 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 pro- enzymes or zymogens and the inhibition of active enzymes by endogenous inhibitors, $\alpha_2$-macroglobulin, and tissue inhibitors of metalloproteinases (TIMPs).

MMP-9 (also referred to as gelatinase B, 92 kDa type IV collagenase, 92 kDa gelatinase, and type V collagenase) is secreted as a glycosylated pro-enzyme (4). Activation of the pro-enzyme involves a proteolytic removal of the N-terminal pro region containing the cysteine switch motif conserved in MMPs (5). The resulting 82 kDa active enzyme consists of a catalytic domain with a zinc-binding motif conserved in metzincins (6, 7). The catalytic domain also contains three contiguous fibronectin type II homology units responsible for binding gelatin (8). A proline-rich hinge region links the catalytic domain to the C-terminal hemopexin-like domain. *In vitro* treatment of the pro-enzyme with 4-aminophenylmercuric acetate (APMA) produces not only the active enzyme but also a C-terminal truncated form with activity comparable to that of the active form (9).

MMP-9 degrades components of the ECM with high specific activity for denatured collagens (gelatin). It can cleave native collagens of type IV, V, and XI, as well as elastin. MMP-9 can also cleave a variety of non-ECM molecules such as IL-1β, IL-8, connective tissue-activating peptide-III, platelet factor-4, GROα, substance P, myelin basic protein, and amyloid β peptide. MMP-9 can increase or decrease the biological activity of these molecules, depending upon the site of cleavage (10, 11).

MMP-9 is produced by a variety of normal and transformed cells including neutrophils, monocytes, macrophages, astrocytes, fibroblasts, osteoclasts, chondrocytes, keratinocytes, endothelial and epithelial cells, and is regulated by various agents. Transgenic mouse models report that MMP-9 regulates growth plate angiogenesis and apoptosis of hypertrophic chondrocytes (12), mediates early bone development through osteoclast recruitment (13), suppresses development of experimental abdominal aortic aneurysms (14), influences sensitization and reward (15), allows cytotrophoblast invasion (16), contributes to skin carcinogenesis (17), triggers the angiogenic switch during carcinogenesis (18), inactivates the serpin α1-proteinase inhibitor (19), and associates with ischemia-induced blood brain barrier permeability (20).

The Quantikine Mouse Total MMP-9 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure total mouse MMP-9 (pro-, active, and TIMP-complexed MMP-9) in cell culture supernates, serum, and platelet-poor plasma. It contains NS0-expressed recombinant mouse MMP-9 and antibodies raised against the recombinant factor. Natural mouse MMP-9 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 mouse MMP-9.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse MMP-9 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any mouse MMP-9 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse MMP-9 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 mouse MMP-9 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.
- For best results, pipette reagents and samples into the center of each well.
- It is recommended that the samples be pipetted within 15 minutes.
- 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.

## 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 MMP-9 Microplate** | 893186 | 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse MMP-9. | 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-9 Standard** | 893733 | 2 vials (10 ng/vial) of recombinant mouse MMP-9 in a buffered protein base with preservatives; lyophilized. | Use a new Standard and Control for each assay. |
| **Mouse Total MMP-9 Control** | 893189 | 2 vials of recombinant mouse MMP-9 in a buffered protein base with preservatives; lyophilized. The concentration range of the mouse MMP-9 after reconstitution is shown on the vial label. The assay value of the Control should be within the range specified on the label. | |
| **Mouse Total MMP-9 Conjugate** | 893187 | 12 mL of a polyclonal antibody against mouse MMP-9 conjugated to horseradish peroxidase with preservatives. | May be stored for up to 1 month at 2-8 °C.* |
| **Assay Diluent RD1-34** | 895265 | 11 mL of a buffered protein base with blue dye and preservatives. | |
| **Calibrator Diluent RD5-10** | 895266 | 2 vials (21 mL/vial) of a buffered protein solution with preservatives. | |
| **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** | 895174 | 23 mL of diluted hydrochloric 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.
- 500 mL graduated cylinder.
- 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 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. 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.

**Platelet-poor Plasma** - Collect plasma on ice using heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. An additional centrifugation step of the plasma at 10,000 x g for 10 minutes at 2-8 °C is recommended for complete platelet removal. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Note:** *EDTA and Citrate plasma are not recommended for use in this assay due to their chelating properties.*

**MMP-9 is present in neutrophil granules and is released upon neutrophil exposure to activated platelets. Therefore, to measure circulating levels of MMP-9, platelet-free plasma should be collected for measurement. It should be noted that many protocols for plasma preparation, including procedures recommended by the Clinical Laboratory and Standards Institute (CLSI), result in incomplete removal of platelets from blood. This will cause variable and irreproducible results for assays of factors released by platelet activation.**

## SAMPLE PREPARATION

Serum samples require a 50-fold dilution. A suggested 50-fold dilution is 10 µL of sample + 490 µL of Calibrator Diluent RD5-10.

Platelet-poor plasma samples require a 20-fold dilution. A suggested 20-fold dilution is 10 µL of sample + 190 µL of Calibrator Diluent RD5-10.

Cell culture supernate samples may require dilution.

## REAGENT PREPARATION

**Bring all reagents to room temperature before use.**

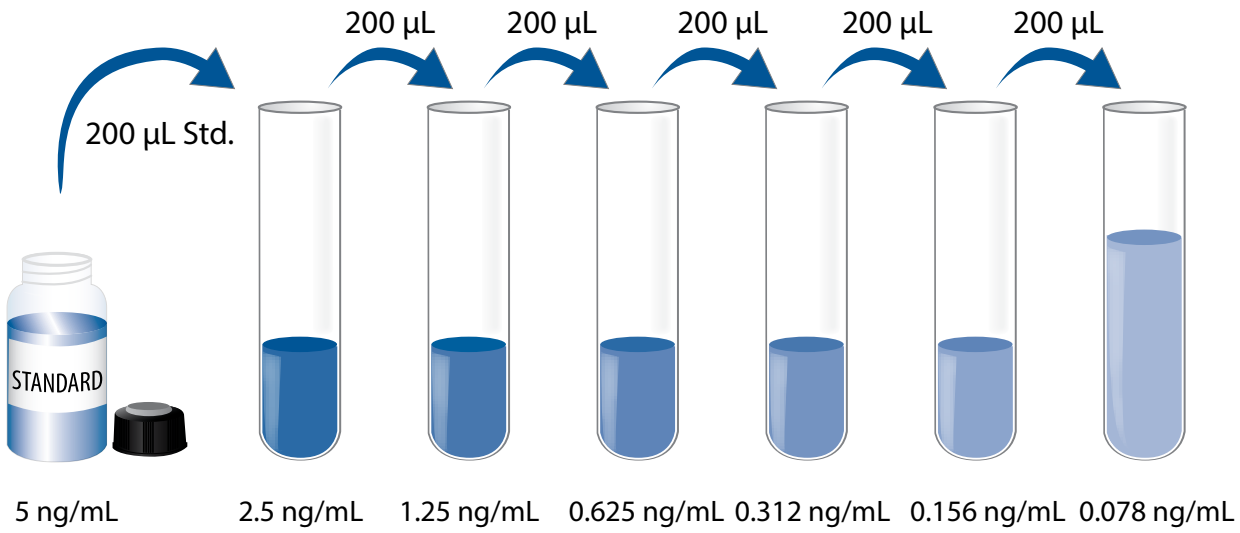
**Mouse Total MMP-9 Control** - Reconstitute the Control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the Control undiluted.

**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 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.

**Mouse Total MMP-9 Standard** - Reconstitute the Mouse Total MMP-9 Standard with 2.0 mL of Calibrator Diluent RD5-10. This reconstitution produces a stock solution of 5 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 RD5-10 into each tube. Use the Stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The 5 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, Control, and samples 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 RD1-34 to each well.
4. Add 50 µL of Standard, Control, or sample* per well. Gently tap the plate to ensure thorough mixing. 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-9 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 100 µL of Stop Solution to each well. 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

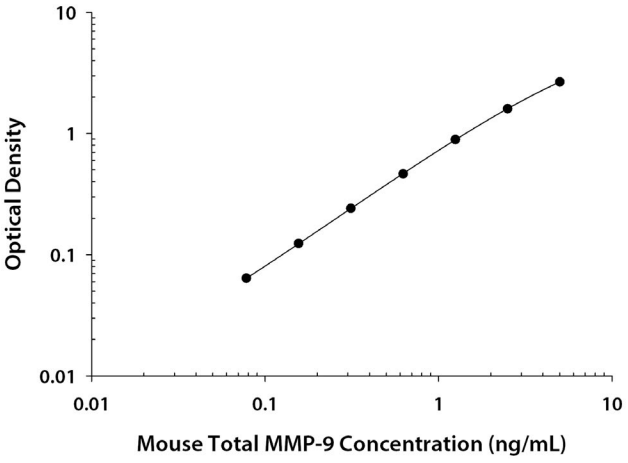
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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-9 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 | 0.031<br>0.033 | 0.032 | — |
| 0.078 | 0.092<br>0.099 | 0.096 | 0.064 |
| 0.156 | 0.153<br>0.158 | 0.156 | 0.124 |
| 0.312 | 0.267<br>0.278 | 0.273 | 0.241 |
| 0.625 | 0.487<br>0.504 | 0.496 | 0.464 |
| 1.25 | 0.917<br>0.932 | 0.925 | 0.893 |
| 2.5 | 1.624<br>1.642 | 1.633 | 1.601 |
| 5 | 2.670<br>2.722 | 2.696 | 2.664 |

## 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)** | 0.239 | 0.731 | 1.71 | 0.211 | 0.600 | 1.61 |
| **Standard deviation** | 0.024 | 0.037 | 0.097 | 0.020 | 0.049 | 0.125 |
| **CV (%)** | 8.4 | 5.1 | 5.7 | 9.6 | 8.1 | 7.8 |

## RECOVERY

The recovery of mouse MMP-9 spiked to 3 levels throughout the range of the assay was evaluated.

| Sample Type | Average % Recovery | Range |
|---|---|---|
| Cell culture samples (n=4) | 94 | 86-108% |

## LINEARITY

To assess the linearity of the assay, samples containing high concentrations of mouse MMP-9 were diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

| | | Cell culture supernates (n=4) | Serum* (n=4) | Platelet-poor Heparin plasma* (n=4) |
|---|---|---|---|---|
| 1:2 | Average % of Expected | 103 | 102 | 106 |
| | Range (%) | 99-108 | 95-106 | 105-109 |
| 1:4 | Average % of Expected | 109 | 110 | 110 |
| | Range (%) | 101-115 | 106-114 | 109-111 |
| 1:8 | Average % of Expected | 110 | 112 | 107 |
| | Range (%) | 105-114 | 108-117 | 96-114 |
| 1:16 | Average % of Expected | 110 | 111 | 107 |
| | Range (%) | 103-115 | 100-117 | 104-109 |

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

## SENSITIVITY

Forty-four assays were evaluated and the minimum detectable dose (MDD) of mouse MMP-9 ranged from 0.003-0.014 ng/mL. The mean MDD was 0.007 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 MMP-9 produced at R&D Systems.

## SAMPLE VALUES

**Serum/Platelet-poor Plasma** - Samples were evaluated for the presence of MMP-9 in this assay.

| Sample Type | Mean (ng/mL) | Range (ng/mL) | Standard Deviation (ng/mL) |
|---|---|---|---|
| Serum (n=20) | 121 | 46.2-223 | 44 |
| Platelet-poor heparin plasma (n=20) | 59 | 19.7-191 | 48 |

**Cell Culture Supernates:**
J774A.1 mouse reticulum cell sarcoma macrophage cells were cultured for one day in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were stimulated with 2.5 µg/mL LPS. An aliquot was removed, tested for natural mouse MMP-9, and measured 46.1 ng/mL.

Mouse lung tissue (3 lungs in 1-2 mm pieces in 100 mL of medium) was cultured for one day in RPMI supplemented with 10% fetal bovine serum, 5 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot was removed, tested for detectable levels of natural mouse MMP-9, and measured 15.6 ng/mL.

Mouse spleen tissue (2 spleens in 1-2 mm pieces in 100 mL of medium) was cultured for four days in RPMI supplemented with 10% fetal bovine serum, 5 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot was removed, and tested for natural mouse MMP-9, and measured 44.3 ng/mL.

P388D1 mouse lymphoma cells were cultured for 3 days in RPMI supplemented with 10% fetal bovine serum and 0.2 mM L-glutamine. An aliquot was removed, tested for natural mouse MMP-9, and measured 4.62 ng/mL.

## SPECIFICITY

This assay recognizes natural and recombinant mouse MMP-9 (pro-, active, and TIMP-complexed).

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant mouse MMP-9 control were assayed for interference. No significant cross-reactivity or interference was observed.

**Recombinant mouse:**

Lipocalin-2/NGAL
MMP-2
MMP-3
MMP-7
MMP-8
MMP-12
TIMP-1

**Recombinant rat:**

MMP-8
MMP-9

**Recombinant human:**

MMP-1
MMP-2
MMP-3
MMP-7
MMP-8
MMP-9
MMP-10
MMP-12
MMP-13
MMP-14 (aa 21-284)
MMP-16 (aa 32-291)

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