# Magnetic Luminex® Performance Assay

## Human MMP Base Kit

Catalog Number LMPM000

For the simultaneous quantitative determination of multiple human matrix metalloproteinase (MMP) concentrations in cell culture supernates, serum, plasma, platelet-poor plasma, saliva, and urine.

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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614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

**DISTRIBUTED BY:**

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19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
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24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

# INTRODUCTION

The matrix metalloproteinases (MMPs) consist of 24 known human zinc proteases with essential roles in breaking down components of the extracellular matrix (ECM) (1-5). In addition to ECM proteins, other potential MMP substrates include cytokines (6-10), chemokines (11), growth factors and binding proteins (12-15), cell/cell adhesion molecules (16), and other proteinases (17, 18). With a few exceptions, MMPs share common structural motifs including a pro-peptide domain, a catalytic domain, a hinge region, and a hemopexin-like domain (2, 4, 5). Synthesized as pro-enzymes, most are secreted before conversion to their active forms. In general, the activation mechanism is thought to occur in a stepwise fashion involving disruption of the interaction between the catalytic site zinc and a cysteine-thiol group in the pro-peptide domain. This is followed by cleavage of the pro-peptide (5). Activation can be mediated by several serine proteases (19-21), MMPs (4, 17, 21-22), or potentially via NO-mediated S-nitrosylation of the pro-peptide cysteine-thiol group (23). In some cases, activation can take place intracellularly via a furin-like serine protease (24, 25). MMPs are expressed by many cell types and can be upregulated in response to adhesion molecules, growth factors, cytokines, and hormones (2-5). They have been implicated in several physiological processes including tissue morphogenesis (26-28), cell migration (29-31), wound healing (32), bone remodeling (33, 34), and angiogenesis (35-37). MMP activities are modulated on several levels including transcription, pro-enzyme activation, or by their endogenous inhibitors, tissue inhibitors of metalloproteinases (TIMPs) (5, 38). Imbalances in MMP regulation have been implicated in several pathological processes including cancer (39, 40), cardiovascular disorders (41, 42), and arthritis (43-45).

MMPs included in this panel:

| Analyte | Catalog Number | Microparticle Region |
|---|---|---|
| EMMPRIN/CD147 | LMPM972 | 30 |
| MMP-1 | LMPM901 | 20 |
| MMP-2 | LMPM902 | 19 |
| MMP-3 | LMPM513 | 21 |
| MMP-7 | LMPM907 | 22 |

| Analyte | Catalog Number | Microparticle Region |
|---|---|---|
| MMP-8 | LMPM908 | 25 |
| MMP-9 | LMPM911 | 26 |
| MMP-10 | LMPM910 | 27 |
| MMP-12 | LMPM919 | 28 |
| MMP-13 | LMPM511 | 29 |

# PRINCIPLE OF THE ASSAY

Magnetic Luminex® Performance Assay multiplex kits are designed for use with the Luminex MAGPIX® CCD Imager. Alternatively, kits can be used with the Luminex 100/200™ or Bio-Rad® Bio-Plex®, dual laser, flow-based sorting and detection platforms.

Analyte-specific antibodies are pre-coated onto color-coded magnetic microparticles. Microparticles, standards and samples are pipetted into wells and the immobilized antibodies bind the analytes of interest. After washing away any unbound substances, a biotinylated antibody cocktail specific to the analytes of interest is added to each well. Following a wash to remove any unbound biotinylated antibody, streptavidin-phycoerythrin conjugate (Streptavidin-PE), which binds to the biotinylated antibody, is added to each well. A final wash removes unbound Streptavidin-PE, the microparticles are resuspended in buffer and read using the Luminex MAGPIX Analyzer. A magnet in the analyzer captures and holds the superparamagnetic microparticles in a monolayer. Two spectrally distinct Light Emitting Diodes (LEDs) illuminate the beads. One LED identifies the analyte that is being detected and the second LED determines the magnitude of the PE-derived signal, which is in direct proportion to the amount of analyte bound. Each well is imaged with a CCD camera. Kits can also be used with Luminex 100/200 or Bio-Rad Bio-Plex dual laser, flow-based systems.

## 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 fall outside the dynamic range of the assay, 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 these factors have been tested in the Luminex Performance Assay, the possibility of interference cannot be excluded.
- Magnetic Luminex Performance Assays afford the user the benefit of multianalyte analysis of biomarkers in a single complex sample. A single multipurpose diluent is used to optimize recovery, linearity, and reproducibility. Such a multipurpose diluent may not optimize any single analyte to the same degree that a unique diluent selected for analysis of that analyte can optimize conditions. Therefore, some performance characteristics may be more variable than those for assays designed specifically for single analyte analysis.
- **Only the analytes listed on the Standard Value Card can be measured with this base kit.**

## 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.
- Protect microparticles and Streptavidin-PE from light at all times to prevent photobleaching.

## PRECAUTIONS

MMPs are detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

## MATERIALS PROVIDED & STORAGE CONDITIONS

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

| PART | PART # | DESCRIPTION | STORAGE OF OPENED, DILUTED, OR RECONSTITUTED MATERIAL |
|---|---|---|---|
| **MMP Panel Standard Cocktail** | 894339 | 2 vials of recombinant human MMPs in a buffered protein base with preservatives; lyophilized. | Discard after use. Use a fresh standard for each assay. |
| **Microparticle Diluent 3** | 895857 | 6 mL of a buffered protein base with preservatives. | May be stored for up to 1 month at 2-8 °C.* *Once diluted, any unused microparticle cocktail must be discarded.* |
| **Biotin Antibody Diluent 2** | 895832 | 5.5 mL of a buffered protein base with preservatives. | May be stored for up to 1 month at 2-8 °C.* |
| **Calibrator Diluent RD5-37** | 895853 | 21 mL of a buffered protein base with preservatives. | |
| **Streptavidin-PE** | 892525 | 0.07 mL of a 100-fold concentrated streptavidin-phycoerythrin conjugate with preservatives. | |
| **Wash Buffer Concentrate** | 895003 | 21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.* | |
| **Microplate** | 641385 | 1 flat-bottomed 96-well microplate used as a vessel for the assay. | |
| **Mixing Bottles** | 895505 | 2 empty 8 mL bottles used for mixing microparticles with Microparticle Diluent. | |
| **Plate Sealers** | 640445 | 4 adhesive foil strips. | |
| **Standard Value Card** | 749407 | 1 card listing the Standard reconstitution volume and working standard concentrations for this lot of base kit. | |

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

## OTHER SUPPLIES REQUIRED

- **Luminex Performance Assay analyte-specific kit(s) (see Introduction on page 1).**
- Luminex MAGPIX, Luminex 100/200, or Bio-Rad Bio-Plex analyzer with X-Y platform.
- Hand-held microplate magnet or platewasher with a magnetic platform.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Multi-channel pipette, manifold dispenser, or automated dispensing unit.
- 50 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 800 ± 50 rpm.
- Microcentrifuge.
- **Polypropylene** test tubes for dilution of standards and samples.

## SAMPLE COLLECTION AND 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.

**Note:** *Cell culture supernate samples are not suitable for use in the MMP-2 assay.*

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

**Platelet-poor Plasma*** - Collect plasma on ice using heparin as an anticoagulant. Centrifuge at 2-8 °C for 15 minutes at 1000 x g within 30 minutes of collection. For complete platelet removal, centrifuge the separated plasma at 10,000 x g for 10 minutes at 2-8 °C. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Note:** *Plasma and platelet-poor plasma samples are not suitable for use in the MMP-13 assay. EDTA and Citrate are not recommended for use in this assay due to their chelating properties. Hemolyzed and icteric samples are not suitable for use in this assay.*

***Some MMPs may be released upon platelet activation. For example, to measure circulating levels of MMP-9, platelet-poor plasma should be used. It should be noted that many protocols for plasma preparation, including procedures recommended by the Clinical and Laboratory Standards Institute (CLSI), result in incomplete removal of platelets or platelet activation. This may cause variable and irreproducible results for assays of factors contained in platelets and released by platelet activation.**

**Saliva** - Collect saliva in a tube and centrifuge for 5 minutes at 10,000 x g. Collect the aqueous layer, and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Urine** - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

## SAMPLE PREPARATION

**Use polypropylene tubes.**

**Note:** *When assaying serum and plasma samples, EMMPRIN cannot be multiplexed with MMP-7, MMP-8, MMP-10, MMP-12, or MMP-13 (R&D Systems Catalog #'s LMPM907, LMPM908, LMPM910, LMPM919, and LMPM511, respectively).*

Cell culture supernate, serum, plasma, and platelet-poor plasma samples require a 5-fold dilution. A suggested 5-fold dilution is 40 µL of sample + 160 µL of Calibrator Diluent RD5-37. Mix thoroughly.

MMP-2, MMP-8, MMP-9, and MMP-12 serum and plasma samples must be further diluted 10-fold to a final 50-fold dilution. A suggested 50-fold dilution is 20 µL of the 5-fold diluted sample + 180 µL of Calibrator Diluent RD5-37. Mix thoroughly.

Saliva samples require a 40-fold dilution. A suggested 40-fold dilution is 10 µL of sample + 390 µL of Calibrator Diluent RD5-37. Mix thoroughly.

Urine samples require a 10-fold dilution. A suggested 10-fold dilution is 15 µL of sample + 135 µL of Calibrator Diluent RD5-37. Mix thoroughly.

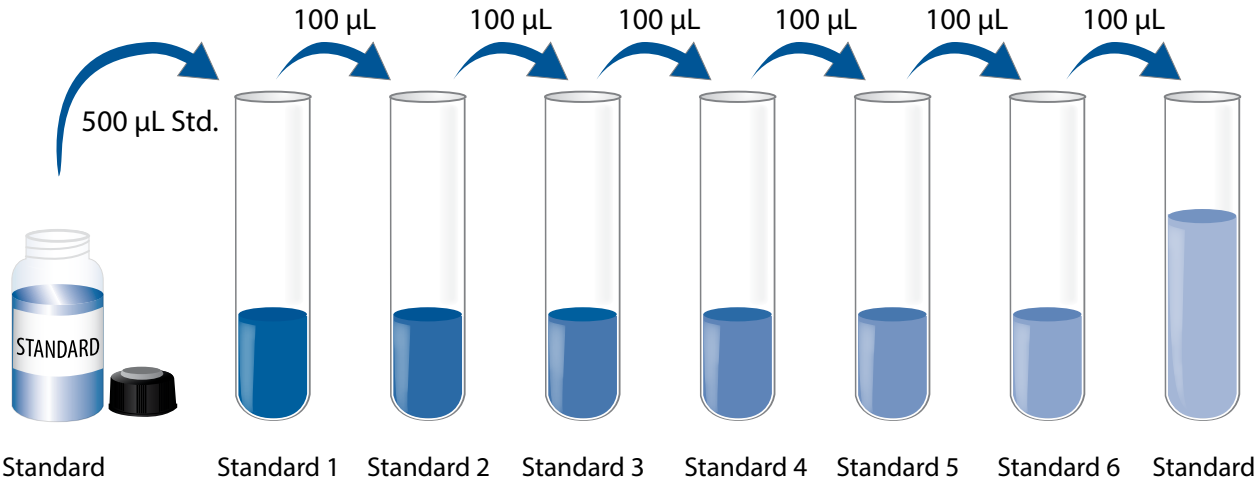
## REAGENT PREPARATION

**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.

**Standard** - Reconstitute the Standard with Calibrator Diluent RD5-37. Refer to the Standard Value Card for the reconstitution volume and assigned values. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

**Use polypropylene tubes.** Pipette 500 µL of the reconstituted Standard into the Standard 1 tube. Pipette 200 µL of Calibrator Diluent RD5-37 into the remaining tubes. Use Standard 1 to produce a 3-fold dilution series (below). *See analyte specific datasheets for details.* Mix each tube thoroughly before the next transfer. Standard 1 serves as the high standard. Calibrator Diluent RD5-37 serves as the blank.



## DILUTED MICROPARTICLE COCKTAIL PREPARATION

1. Centrifuge each Microparticle Concentrate vial for 30 seconds at 1000 x g prior to removing the cap.
2. Gently vortex the vials to resuspend the microparticles, taking precautions not to invert the vials.
3. Dilute the Microparticle Concentrates in the mixing bottle provided. The volume of the Microparticle Concentrate listed in the table below is for each analyte (*e.g.* if measuring a full plate of MMP-1 and MMP-9, add 50 µL of MMP-1 Microparticle Concentrate and 50 µL of MMP-9 Microparticle Concentrate to 5 mL of Microparticle Diluent 3).

| Number of Wells Used | Microparticle Concentrate | + | Microparticle Diluent 3 |
|---|---|---|---|
| 96 | 50.0 µL | + | 5.00 mL |
| 72 | 37.5 µL | + | 3.75 mL |
| 48 | 25.0 µL | + | 2.50 mL |
| 24 | 12.5 µL | + | 1.25 mL |

**Note:** *Protect microparticles from light during handling. Diluted microparticles cannot be stored. Prepare microparticles within 30 minutes of use.*

## DILUTED BIOTIN ANTIBODY COCKTAIL PREPARATION

1. Centrifuge each Biotin Antibody Concentrate vial for 30 seconds at 1000 x g prior to removing the cap.
2. Gently vortex the vials, taking precautions not to invert the vials.
3. Add 50 µL of each Biotin Antibody Concentrate to the vial of Biotin Antibody Diluent 2. Mix gently.

## STREPTAVIDIN-PE PREPARATION

**Use a polypropylene amber bottle or a polypropylene tube wrapped with aluminum foil. Protect Streptavidin-PE from light during handling and storage.**

1. Centrifuge the Streptavidin-PE vial for 30 seconds at 1000 x g prior to removing the cap.
2. Gently vortex the vial, taking precautions not to invert the vial.
3. Dilute the 100X Streptavidin-PE to a 1X concentration by adding 55 µL of Streptavidin-PE to 5.5 mL of Wash Buffer.

## INSTRUMENT SETTINGS

**Luminex MAGPIX analyzer:**

a) Assign the microparticle region for each analyte being measured (see Introduction on page 1)

b) 50 events/bead

c) Sample size: 50 µL

d) Collect Median Fluorescence Intensity (MFI)

**Luminex 100/200 and Bio-Rad Bio-Plex analyzers:**

**Note:** *Calibrate the analyzer using the proper reagents for superparamagnetic microparticles (refer to instrument manual).*

a) Assign the bead region for each analyte being measured (see Introduction on page 1)

b) 50 events/bead

c) Minimum events: 0

d) Flow rate: 60 µL/minute (fast)

e) Sample size: 50 µL

f ) Doublet Discriminator gates at approximately 8000 and 16,500

g) Collect MFI

**Note:** *The CAL2 setting for the Bio-Rad Bio-Plex analyzer should be set at the low RP1 target value.*

## ASSAY PROCEDURE

**Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.**

**Note:** *Protect microparticles and Streptavidin-PE from light at all times.*
*MMPs are detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.*

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Resuspend the diluted microparticle cocktail by inversion or vortexing. Add 50 µL of the microparticle cocktail to each well of the microplate.
3. Add 50 µL of Standard or sample* per well. Pipette assay within 15 minutes. Securely cover with a foil plate sealer. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 800 ± 50 rpm.
4. Using a magnetic device designed to accommodate a microplate, wash by applying the magnet to the bottom of the microplate, removing the liquid, filling each well with Wash Buffer (100 µL) and removing the liquid again. Complete removal of liquid is essential for good performance. Perform the wash procedure three times.

   **Note:** *Refer to the magnetic device user manual for proper wash technique using a round bottom microplate.*
5. Add 50 µL of diluted Biotin Antibody Cocktail to all wells. Securely cover with a foil plate sealer and incubate for 1 hour at room temperature on the shaker set at 800 ± 50 rpm.
6. Repeat the wash as in step 4.
7. Add 50 µL of diluted Streptavidin-PE to all wells. Securely cover with a foil plate sealer and incubate for 30 minutes at room temperature on the shaker set at 800 ± 50 rpm.
8. Repeat the wash as in step 4.
9. Resuspend the microparticles by adding 100 µL of Wash Buffer to each well. Incubate for 2 minutes on the shaker set at 800 ± 50 rpm.
10. Read within 90 minutes using the Luminex or Bio-Rad analyzer.
    **Note:** *Resuspend microparticles immediately prior to reading.*

*Samples require dilution. See Sample Preparation section.

## CALCULATION OF RESULTS

Use the Standard concentrations on the Standard Value Card and calculate 3-fold dilutions for the remaining levels. Average the duplicate readings for each standard and sample and subtract the average blank Median Fluorescence Intensity (MFI).

Create a standard curve for each analyte by reducing the data using computer software capable of generating a five parameter logistic (5-PL) curve-fit.

Since samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## CALIBRATION

This assay is calibrated against highly purified recombinant human MMPs produced at R&D Systems.

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