

# Fluorokine<sup>®</sup> MAP

## Human MMP MultiAnalyte Profiling Base Kit

Catalog Number LMP000

**For the simultaneous quantitative determination of the concentrations of multiple human matrix metalloproteinases (MMPs) 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

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 form. 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), 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:

<b>MMP</b>	<b>Alternative Enzyme Name</b>	<b>MMP</b>	<b>Alternative Enzyme Name</b>
MMP-1	Collagenase 1	MMP-8	Collagenase 2
MMP-2	Gelatinase A	MMP-9	Gelatinase B
MMP-3	Stromelysin 1	MMP-12	Macrophage Metalloelastase
MMP-7	Matrilysin	MMP-13	Collagenase 3

## PRINCIPLE OF THE ASSAY

Fluorokine<sup>®</sup> MAP multiplex kits are designed for use with the Luminex<sup>®</sup> 100™, Luminex 200™, or Bio-Rad<sup>®</sup> Bio-Plex<sup>®</sup> dual laser, flow-based sorting and detection analyzers manufactured by Luminex Corporation.

Analyte-specific antibodies are pre-coated onto color-coded microparticles. Microparticles, standards and samples are pipetted into wells and the immobilized antibodies bind the analytes of interest. After washing away any unbound substances, biotinylated antibodies specific to the analytes of interest are added to each well. Following a wash to remove any unbound biotinylated antibody, streptavidin-phycoerythrin conjugate (Streptavidin-PE), which binds the biotinylated detection antibodies, is added to each well. A final wash removes unbound Streptavidin-PE and the microparticles are resuspended in buffer and read using the Luminex analyzer. One laser is microparticle-specific and determines which analyte is being detected. The other laser determines the magnitude of the phycoerythrin-derived signal, which is in direct proportion to the amount of analyte bound.

## 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.
- This assay is designed to eliminate interference by other enzymes and proteins present in biological samples. Until all factors have been tested in the Fluorokine MAP assay, the possibility of interference cannot be excluded.
- Fluorokine MAP affords the user the benefit of multianalyte analysis of MMPs in a complex sample. A single, multipurpose diluent is used to optimize recovery, linearity and reproducibility. Such a multipurpose, single 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 analytes listed on the enclosed Standard Value Card can be measured with this base kit.**

## MATERIALS PROVIDED

**Standard MMP Cocktail** (Part 895851) - 2 vials of recombinant human MMPs in a buffered protein base with preservatives; lyophilized.

**Standard Value Card** (Part 750076) - 1 card listing the standard reconstitution volume and concentrations for this lot of base kit.

**Microparticle Diluent 3** (Part 895857) - 6 mL of a buffered protein base with preservatives.

**Calibrator Diluent RD5-37** (Part 895853) - 21 mL of buffered protein base with preservatives.

**Wash Buffer Concentrate** (Part 895003) - 21 mL of a 25-fold concentrated solution of buffered surfactant with preservative.

**Biotin Antibody Diluent 2** (Part 895832) - 5.25 mL of a buffered protein base with preservative.

**Streptavidin-PE** (Part 892525) - 0.07 mL of a 100-fold concentrated streptavidin-phycoerythrin conjugate with preservatives.

**Microplate** (Part 640763) - 1 filter-bottomed plate, used as a vessel for the assay.

**Plate Covers** (Part 640445) - 4 adhesive strips.

**Mixing Bottles** (Part 895505) - 2 (8 mL) empty bottles used for mixing microparticles with Microparticle Diluent.

## STORAGE

<b>Unopened Kit</b>	Store at 2-8° C. Do not use past kit expiration date.	
<b>Opened/ Reconstituted Reagents</b>	Diluted Wash Buffer	May be stored for up to 1 month at 2-8° C.*
	Biotin Antibody Diluent 2	
	Calibrator Diluent RD5-37	
	Microparticle Diluent 3	
	Diluted Streptavidin-PE	
	Biotin Antibody Cocktail	
	Diluted Microparticle Mixture	Discard any unused diluted microparticle mixture.
	Standard	Discard after use. Use a fresh standard for each assay.

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

## OTHER SUPPLIES REQUIRED

- **Fluorokine MAP analyte-specific kit(s). See the Appendix on page 11 for a complete list of products that can be used with this base kit.**
- Luminex 100, Luminex 200, or Bio-Rad Bio-Plex Analyzer with X-Y platform.
- Microplate vacuum manifold (Millipore Multiscreen™ Vacuum Manifold, Catalog # MAVM096, or equivalent).
- 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 500 ± 50 rpm.
- Microcentrifuge.
- **Polypropylene** test tubes for dilution of standards and samples.

## SAMPLE COLLECTION AND STORAGE

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

**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 National Committee for Clinical Laboratory Standards (NCCLS), 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.**

## SAMPLE PREPARATION

Serum/plasma samples require a 10-fold dilution. A suggested 10-fold dilution is 15  $\mu\text{L}$  of sample + 135  $\mu\text{L}$  of Calibrator Diluent RD5-37. Mix thoroughly.

When assaying MMP-9, serum and plasma samples must be further diluted 10-fold to a final 100-fold dilution. A suggested 100-fold dilution is 15  $\mu\text{L}$  of the 10-fold diluted sample + 135  $\mu\text{L}$  of Calibrator Diluent RD5-37. Mix thoroughly.

Platelet-poor plasma samples (for all analytes) require a 10-fold dilution. A suggested 10-fold dilution is 15  $\mu\text{L}$  of sample + 135  $\mu\text{L}$  of Calibrator Diluent RD5-37. Mix thoroughly.

Cell culture media samples require a 5-fold dilution. A suggested 5-fold dilution is 25  $\mu\text{L}$  of sample + 100  $\mu\text{L}$  of Calibrator Diluent RD5-37. Mix thoroughly.

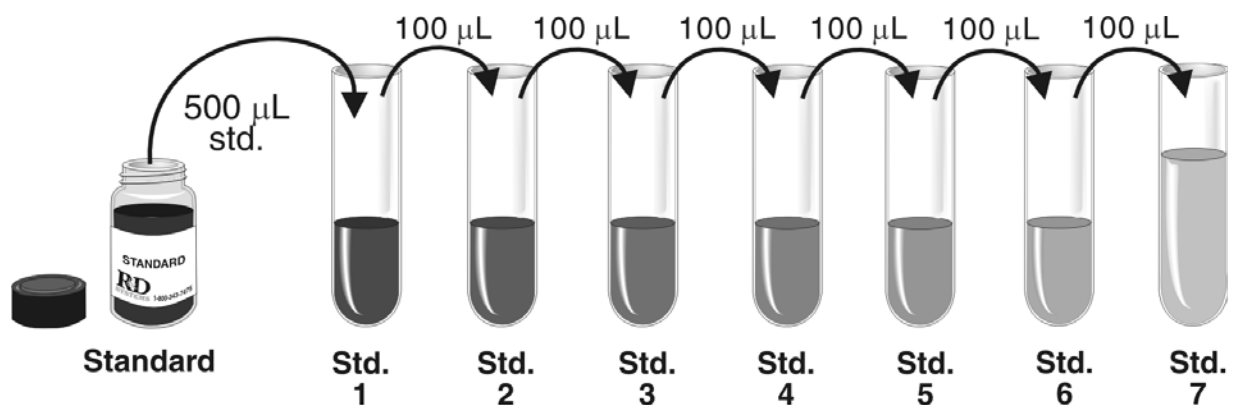
## REAGENT PREPARATION

**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. Dilute 20 mL of Wash Buffer Concentrate into 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  $\mu\text{L}$  of the reconstituted Standard into the Standard 1 tube. Pipette 200  $\mu\text{L}$  of Calibrator Diluent into the remaining tubes. Use Standard 1 to produce a 3-fold dilution series (below). Mix each tube thoroughly before the next transfer. Standard 1 serves as the high standard. The Calibrator Diluent serves as the blank.



## DILUTED MICROPARTICLE 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  $\mu$ L of MMP-1 Microparticle Concentrate and 50  $\mu$ L of MMP-9 Microparticle Concentrate to 5 mL of Microparticle Diluent).

# Wells used	Microparticle Concentrate	+	Microparticle Diluent
96	50 $\mu$ L	+	5.0 mL
72	37.5 $\mu$ L	+	3.75 mL
48	25 $\mu$ L	+	2.5 mL
24	12.5 $\mu$ L	+	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 PREPARATION

1. Centrifuge each Biotin Antibody 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  $\mu$ L of each Biotin Antibody concentrate to the vial of the Biotin Antibody Diluent. Mix gently.

## STREPTAVIDIN-PE PREPARATION

**Use a polypropylene amber bottle or a polypropylene tube covered 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  $\mu$ L of Streptavidin-PE to 5.5 mL of 1X Wash Buffer.

## INSTRUMENT SETTINGS

Adjust the probe height setting on the Luminex analyzer to avoid puncturing the membrane. Refer to the instrument manual.

- a) Assign a bead region for each analyte being measured (refer to page 11)
- b) 50 events/bead
- c) Minimum events: 0
- d) Flow rate: 60  $\mu$ L/Min (fast)
- e) Sample size: 50  $\mu$ L
- f) Doublet Discriminator gates at approximately 7500 and 15500
- g) Collect Median Fluorescence Intensity (MFI)

**Note:** *For the Bio-Rad Bio-Plex analyzer, set the gates at 4300 and 10000. The CAL2 setting for the Bio-Plex analyzer should be set at the low RPI 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.*

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Pre-wet the filter-bottomed microplate by filling each well with 100  $\mu$ L of Wash Buffer. Remove the liquid through the filter at the bottom of the plate using a vacuum manifold designed to accommodate a microplate.
3. Resuspend the diluted microparticle mixture by inversion or vortexing. Add 50  $\mu$ L of the microparticle mixture to each well of the filter-bottomed microplate provided.
4. Add 50  $\mu$ L 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  $500 \pm 50$  rpm.
5. Using a vacuum manifold device designed to accommodate a microplate, wash by removing the liquid, filling each well with Wash Buffer (100  $\mu$ L) and removing the liquid again. All of the liquid must be removed through the filter at the bottom of the plate to avoid any loss of microparticles. Complete removal of liquid is essential for good performance. Perform the wash procedure three times.
6. Add 50  $\mu$ L 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  $500 \pm 50$  rpm.
7. Repeat the wash as in step 5.
8. Add 50  $\mu$ L 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  $500 \pm 50$  rpm.
9. Repeat the wash as in step 5.
10. Resuspend the microparticles by adding 100  $\mu$ L of Wash Buffer to each well. Incubate for 2 minutes on the shaker set at  $500 \pm 50$  rpm.
11. Read within 90 minutes using the Luminex or Bio-Rad analyzer.

\*Samples require dilution. See Sample Preparation section.

# ASSAY PROCEDURE SUMMARY

1. Prepare reagents, samples, and standards as instructed.



2. Pre-wet the plate by filling each well with 100  $\mu$ L of Wash Buffer. Remove the liquid through the filter at the bottom of the plate using a vacuum manifold designed to accommodate a microplate.



3. Add 50  $\mu$ L of diluted microparticle mixture to each well.



4. Add 50  $\mu$ L Standard or sample\* to each well. Incubate 2 hours at RT on a horizontal orbital microplate shaker.



5. Wash by removing the liquid from each well, filling each well with Wash Buffer (100  $\mu$ L), and removing the liquid again. Perform the wash 3 times.



6. Add 50  $\mu$ L diluted Biotin Antibody Cocktail to all wells. Incubate 1 hour at RT on the shaker.



7. Repeat the wash as in step 5.



8. Add 50  $\mu$ L diluted Streptavidin-PE to all wells. Incubate 30 minutes at RT on the shaker.



9. Repeat the wash as in step 5.



10. Add 100  $\mu$ L Wash Buffer to each well. Incubate 2 minutes at RT on the shaker.



11. Read within 90 minutes using a Luminex or Bio-Rad analyzer. Refer to the Instrument Settings section.

**Protect microparticles and Streptavidin-PE  
from light at all times.**

\*Samples require dilution. See Sample Preparation.

## **CALCULATION OF RESULTS**

Use the Standard concentration 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 MFI.

Create a standard curve for each analyte by reducing the data using computer software capable of generating a 5-PL curve fit. As an alternative, construct a standard curve by plotting the MFI 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 MMP concentrations versus the log of the MFI and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

To determine the MMP concentration of each sample, first find the MFI value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding MMP concentration.

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

## **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.
- For best results, adjust the vacuum strength to between 15 and 40 cm of mercury.

## **CALIBRATION**

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

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## APPENDIX

The following products may be used in conjunction with this base kit.

Analyte	Catalog Number	Microparticle Region
MMP-1	LMP901	04
MMP-2	LMP902B	13
MMP-3	LMP513	26
MMP-7	LMP907	30
MMP-8	LMP908	43
MMP-9	LMP911	47
MMP-12	LMP919	62
MMP-13	LMP511	66

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