

Acute Phase Response

Cancer

Cardiovascular Disease

Cytokines Chemokines,  
Growth Factors

Neurology

Toxicology

Infectious Disease

Immunoglobulin Isotyping

Signal Transduction

# Bio-Plex Pro™ Human MMP and TIMP Assays

MAGNETIC SEPARATION ENABLED

MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-12, MMP-13, TIMP-1, TIMP-2, TIMP-3, TIMP-4

- All-in-one MMP 9-plex panel
- All-in-one TIMP 4-plex panel
- Custom configurations
- Single-level quality control
- Magnetic workflow



## High-Performance Multiplex Immunoassays for Research

Bio-Plex Pro human matrix metalloproteinase (MMP) and tissue inhibitors of metalloproteinase (TIMP) assays feature nine MMP and four TIMP magnetic bead-based immunoassays for use in the investigation of many disease pathways. MMPs function in the extracellular environment of cells and degrade both matrix and nonmatrix proteins. MMP activities are regulated by activation of their natural inhibitors, the TIMPs. MMPs have many different functions and actions in the body, while TIMPs work to inhibit the actions of MMPs along with other biological inhibitors. Researchers investigate MMPs and TIMPs together, as the balance between the markers is crucial in determining the status of patient health. MMPs are typically grouped based on historical assessment of their substrate specificity and on their cellular localization (Table 1).

MMPs and TIMPs are associated with the following areas of research:

- Cancer
- Cardiovascular disease
- Infectious disease
- Autoimmune disease
- Neurology

MMPs and TIMPs are markers associated with biological pathways such as:

- Inflammation
- Metastasis
- Apoptosis
- Angiogenesis
- Tumor invasion
- Wound healing

## Assay Features

- Magnetic beads for simplified plate processing
- Single-level quality control with lot-specific ranges
- Assay quick guide to get you started fast
- Compatible with the Bio-Plex® 200 and Bio-Plex 3D suspension array systems and the Bio-Plex® MAGPIX™ multiplex reader

## Rigorous Assay Validation

All Bio-Plex Pro assays undergo a rigorous evaluation that includes the following parameters:

- Specificity and cross-reactivity
- Accuracy (recovery) in key sample matrices
- Inter- and intra-assay precision
- Sensitivity
- Assay working range
- Linearity of dilution
- Parallelism and matrix effect
- Performance characteristics in real samples

Table 1. Classification of MMPs and TIMPs.

Collagenase	Gelatinase	Matriysin	Metalloelastase	Stromelysin	Inhibitors
MMP-1	MMP-2	MMP-7	MMP-12	MMP-3	TIMP-1
MMP-8	MMP-9			MMP-10	TIMP-2
MMP-13					TIMP-3
					TIMP-4



## Assay Performance Definitions

The following parameters are indicative of assay performance as shown in Table 2.

**Assay working range** — the range of concentrations within which the assay is precise and accurate. Boundaries of the assay working range are defined by the lower limit of quantification (LLOQ) and the upper limit of quantification (ULOQ)

**Precision** — the coefficient of variation (%CV) at concentrations within the assay working range

**Accuracy (recovery)** — percentage of the observed concentration relative to the expected concentration of a known amount of analyte within the assay working range

**Sensitivity (limit of detection, LOD)** — the concentration of analyte for which the fluorescence intensity signal is two standard deviations above the background signal

**Table 2. Representative performance characteristics.**

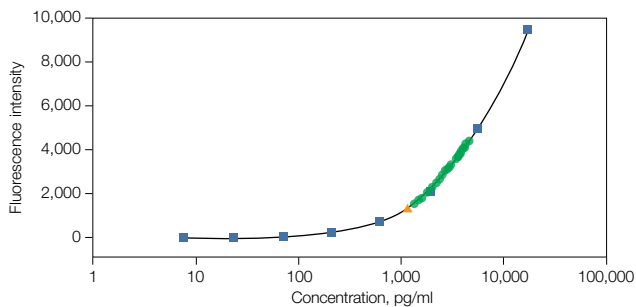
Target	Bead Region	Assay Working Range, pg/ml		Assay Sensitivity, pg/ml
		LLOQ	ULOQ	LOD
<b>Matrix Metalloproteinases</b>				
MMP-1	43	51	111,152	35
MMP-2	26	621	1,357,988	450
MMP-3	45	130	221,609	116
MMP-7	66	21	44,953	5.4
MMP-8	37	30	66,000	1.5
MMP-9	55	138	302,498	24
MMP-10	62	69	83,333	1.6
MMP-12	53	12	25,655	1.0
MMP-13	47	17	38,240	4.9
<b>Tissue Inhibitors of Metalloproteinases</b>				
TIMP-1	21	18.3	19,259	1.6
TIMP-2	64	17.9	39,038	2.4
TIMP-3	52	228.7	13,235	96.3
TIMP-4	35	6.9	15,000	1.7

The LLOQ, ULOQ, LOD, and inter-assay precision %CV (not shown) are mean data determined from three independent multiplex assays in a serum-based matrix. Intra-assay %CV is derived from one representative assay. LLOQ and ULOQ are defined as the boundary standard curve points that meet performance specifications of 10% intra-assay CV and 70–130% recovery. The specification for inter-assay precision <15% is within the assay working range. Data were generated using the magnetic workflow with the Bio-Plex Pro wash station.

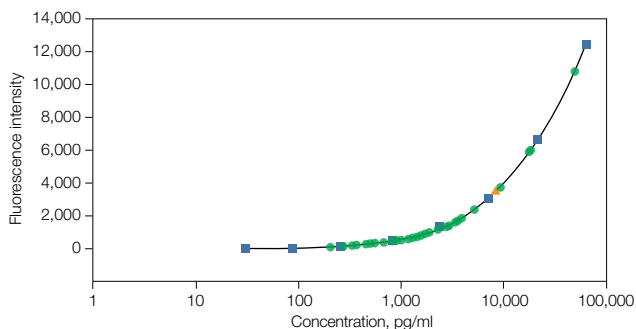
## Bio-Plex Pro Assay Working Range

The assay working range should encompass the biological range of expression in order to be useful in research. Bio-Plex Pro assays are developed and optimized to ensure real sample data fall within the quantifiable regions of the assay as demonstrated by comparing the standard curves of assay controls to biological samples (Figure 1).

### TIMP-2



### MMP-8



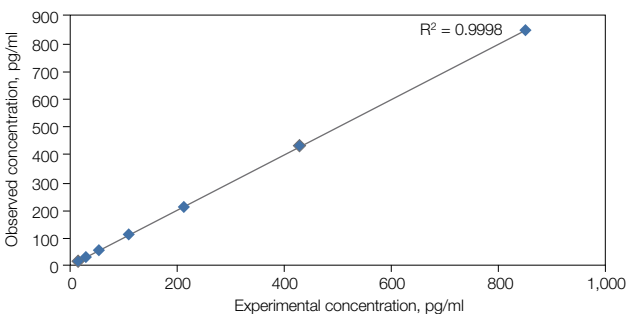
**Fig. 1. Standard curves with assay controls and serum samples.**

Standard points were prepared by serially diluting a reconstituted standard threefold to generate an eight-point standard curve. Standard points with % recovery (■); controls (▲); and samples (●). Data were generated in Bio-Plex Manager™ software and analyzed with Bio-Plex Data Pro™ software.

## Accuracy of Bio-Plex Pro Human MMP and TIMP Assays

Linearity of dilution displays the ability of an assay to generate measured values from complex samples by comparing the accuracy over a range of sample dilutions. Bio-Plex assays are designed and validated with high linearity to yield accurate results from complex matrices (Figure 2).

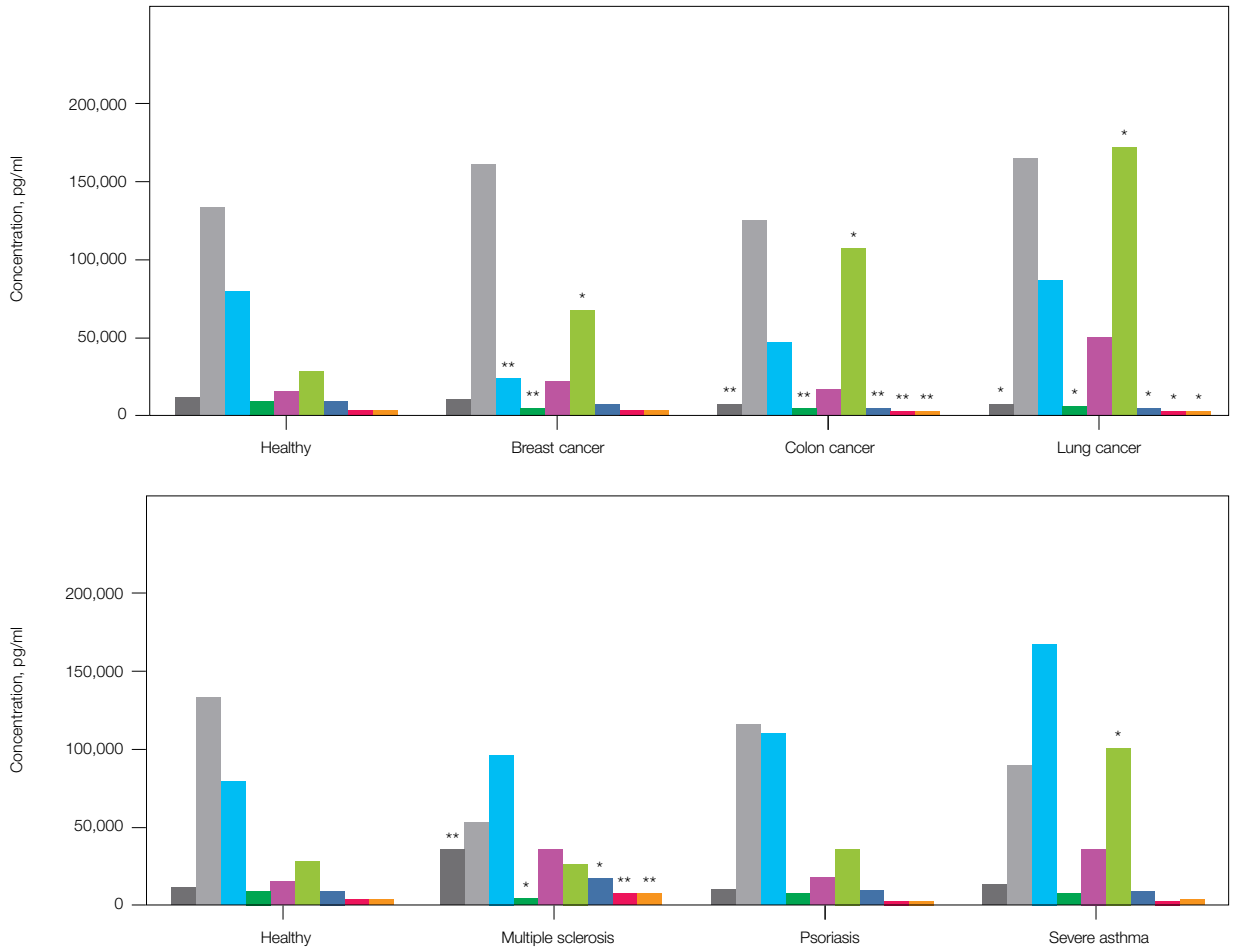
### TIMP-4 (Plasma)



**Fig. 2. Linearity of dilution.** Linearity of dilution was assessed by spiking a known quantity of recombinant antigen into human serum and plasma matrices. Observed and expected analyte concentrations were plotted and the correlation coefficient ( $R^2$ ) values reflect linearity in signal response.

### Detection of Analytes

MMP and TIMP levels can indicate changes in biological pathways associated with diseases. Bio-Plex Pro multiplex assays allow for detection of nine MMP targets measured simultaneously in the same sample. These assays are tested with samples from multiple sources to ensure target analytes are detected within the biological expression levels in normal tissue and levels associated with disease (Figure 3).



**Fig. 3.** Levels of MMPs measured from plasma samples of normal and various disease groups (healthy n = 16, breast cancer n = 19, colon cancer n = 20, lung cancer n = 20, multiple sclerosis n = 12, psoriasis n = 12, and asthma n = 12). Data analysis, graphing, and statistics were performed with Bio-Plex Data Pro software. MMP-1 (■); MMP-2 (■); MMP-3 (■); MMP-7 (■); MMP-8 (■); MMP-9 (■); MMP-10 (■); MMP-12 (■); MMP-13 (■). \*  $P < 0.05$ ; \*\*  $P < 0.01$ .

## Ordering Information

Catalog #	Description	Catalog #	Description
171-AM001M	<b>Bio-Plex Pro Human MMP Panel, 9-Plex,</b> 1 x 96-well, includes coupled magnetic capture beads, premixed detection antibodies, standards, quality controls, detection antibody diluent HB, standard diluent HB, sample diluent HB, assay buffer, wash buffer, streptavidin-PE, 96-well flat bottom plate, sealing tape, and instructions for the detection of MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-12, and MMP-13	<b>Wash Stations and Accessories</b> 300-34376	<b>Bio-Plex Pro Wash Station</b> , microplate wash station for magnetic bead-based assays, includes magnetic plate carrier, waste bottle, and 2 liquid bottles <b>Bio-Plex Handheld Magnetic Washer</b> , includes magnetic washer and adjustment hex tools for use in manual wash steps for all Bio-Plex magnetic assays
171-AM002M	<b>Bio-Plex Pro Human TIMP Panel, 4-Plex,*</b> 1 x 96-well, includes coupled magnetic capture beads, premixed detection antibodies, standards, quality controls, detection antibody diluent HB, diluent HD, assay buffer, wash buffer, streptavidin-PE, 96-well flat bottom plate, sealing tape, and instructions for the detection of TIMP-1, TIMP-2, TIMP-3, and TIMP-4	171-020100	<b>Bio-Plex Pro Flat Bottom Plates</b> , pkg of 40, 96-well plates, for use with Bio-Plex Pro wash stations when using magnetic bead-based assays
<b>Bio-Plex Pro Human MMP Singleplex Sets**</b>		171-025001	<b>Software</b> 171-001510
171-BM001M	Bio-Plex Pro Human MMP-1	171-001513	<b>Bio-Plex Data Pro Software with Bio-Plex Manager Software</b> , Bio-Plex Data Pro software (5 seats), for multi-experiment analysis and advanced data visualization, and Bio-Plex Manager software (5 seats), for instrument data evaluation and optimization. CDs and security HASP key included
171-BM002M	Bio-Plex Pro Human MMP-2	171-STND01	<b>Bio-Plex Data Pro Software</b> , (5 seats), for multi-experiment analysis and advanced data visualization
171-BM003M	Bio-Plex Pro Human MMP-3		<b>Bio-Plex Manager Software</b> , includes 1 user desktop license, for analysis of Bio-Plex data and generation of protocols, does not operate the instrument
171-BM004M	Bio-Plex Pro Human MMP-7		<b>Bio-Plex Manager MP Software Upgrade</b> , for Luminex MAGPIX readers
171-BM005M	Bio-Plex Pro Human MMP-8		
171-BM006M	Bio-Plex Pro Human MMP-9		
171-BM007M	Bio-Plex Pro Human MMP-10		
171-BM008M	Bio-Plex Pro Human MMP-12		
171-BM009M	Bio-Plex Pro Human MMP-13		
<b>Reagent Kits</b>			
171-304090M	<b>Bio-Plex Pro Reagent Kit III</b> , 1 x 96-well, includes detection antibody diluent HB, standard diluent HB, sample diluent HB, assay buffer, wash buffer, streptavidin-PE, flat bottom plate, and sealing tape for magnetic separation methods		* TIMP-3 assay is not intended for use with serum and plasma samples.
<b>Standards</b>			** Singleplex sets include coupled magnetic beads and detection antibodies. Singleplex sets require Bio-Plex Pro reagent kit III (171-304090M) and Bio-Plex Pro human MMP standard (171-DM0001) to run an assay.
171-DM0001	<b>Bio-Plex Pro Human MMP Standard</b> , 1 pkg of 1 vial, lyophilized mixture of 9 standard analytes: MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-12, and MMP-13		The Bio-Plex suspension array system includes fluorescently labeled microspheres and instrumentation licensed to Bio-Rad Laboratories, Inc. by the Luminex Corporation.
171-DM0501	<b>Bio-Plex Pro Human MMP Standard</b> , pkg of 50 lot-matched vials, lyophilized mixture of 9 standard analytes: MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-12, and MMP-13		MAGPIX is a trademark of Luminex Corporation. HASP is a trademark of Aladdin Knowledge Systems, Ltd.



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