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Certificate of Analysis

Ver2 Rev. 1008 LKJ

Catalog Number: 72137-3
Kit Lot Number: *Web version; not Lot-specific]*
Size: 96 Tests

Human MMP Panel Assays	xMAP® Region
• MMP-1	# 40
• MMP-3	# 41
• MMP-8	# 44
• MMP-9	# 45
• MMP-10	# 46
• MMP-13	# 47

WideScreen™ Human MMP Panel
(Matrix Metalloproteinases)

Description and Use:

The WideScreen™ Human MMP Panel is a pre-mixed multiplex bead kit of quantitative antibody-based assays for simultaneous detection of six matrix metalloproteinases (MMPs) found in biological fluids: MMP-1, MMP-3, MMP-8, MMP-9, MMP-10, and MMP-13. This kit recognizes latent (pro), active, and TIMP-bound forms of the human MMPs included in this panel. The kit includes all the reagents and buffers needed to analyze the above proteins in serum, plasma, and tissue culture supernatants using the Luminex® xMAP® System.

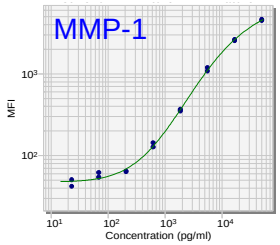
For information on the components and use of this kit, please refer to the WideScreen Human MMP Panel User Protocol (TB528), available at www.novagen.com/WideScreen.
For *in vitro* research use only. Do not use for diagnostic procedures.

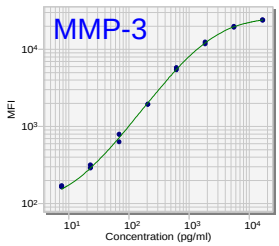
Storage and Stability: Store kit at 2-8°C in the dark. Stable for 6 months from shipment date.

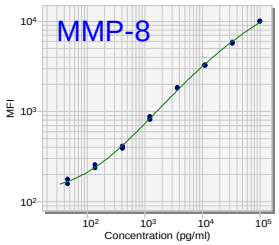
Species Specificity: Human; other species not tested.

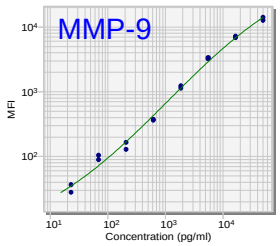
Standard Curves and Concentrations:

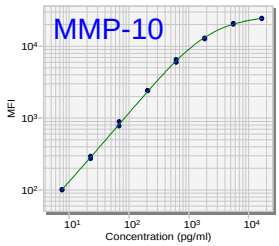
Note: Typical standard curves are shown (not Lot-specific). Concentration is plotted against median fluorescence intensity (MFI) using a five parameter logistic for curve fitting. For accurate quantitation, standard curves must be generated each time an assay is performed.

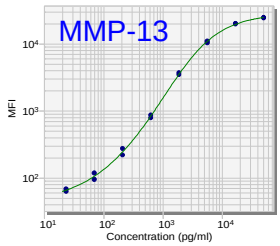












MMP-1		MMP-3		MMP-8		MMP-9		MMP-10		MMP-13	
Conc. (pg/ml)	**MFI**	**Conc.** (pg/ml)	**MFI**	**Conc.** (pg/ml)	**MFI**	**Conc.** (pg/ml)	**MFI**	**Conc.** (pg/ml)	**MFI**	**Conc.** (pg/ml)	**MFI**
50000	4564	**16667**	24040	**100000**	10037	**50000**	13375	**16667**	24376	**50000**	24658
16667	2589	**5556**	19632	**33333**	5774	**16667**	6997	**5556**	20516	**16667**	20149
5556	1138	**1852**	12097	**11111**	3264	**5556**	3294	**1852**	12768	**5556**	10730
1852	359	**617**	5612	**3704**	1831	**1852**	1182	**617**	6252	**1852**	3617
617	135	**206**	1944	**1235**	847	**617**	368	**206**	2408	**617**	835
206	64	**69**	712	**412**	401	**206**	147	**69**	832	**206**	249
69	58	**23**	305	**137**	246	**69**	97	**23**	283	**69**	108
23	46	**8**	168	**46**	167	**23**	32	**8**	101	**23**	66

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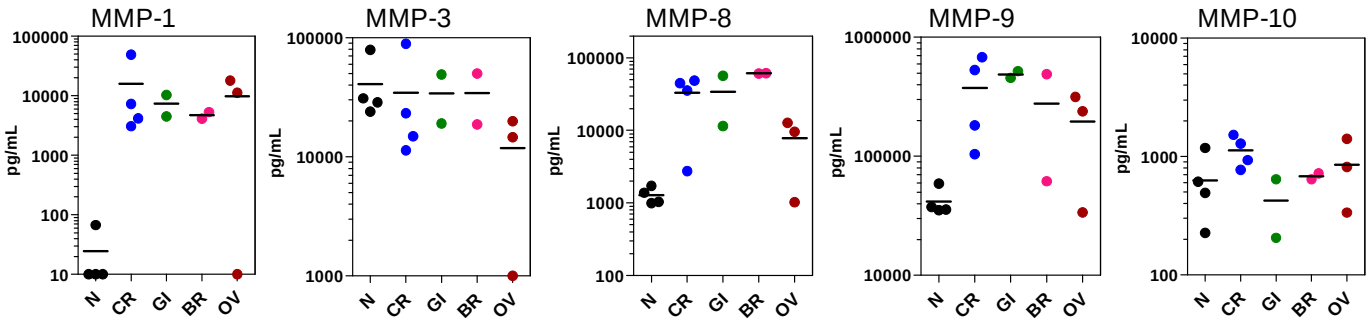
Representative Data:

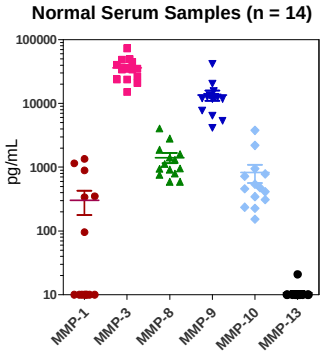
MMP Levels in Human Serum Samples

The WideScreen™ Human MMP Panel was used to quantify MMP levels in randomly selected serum samples from normal and disease state patients: Normal (N, n = 4); Colorectal cancer (CR, n = 4); Gastrointestinal cancer (GI, n = 2); Breast cancer (BR, n = 2); Ovarian cancer (OV, n = 3). Sera from normal individuals have matrix metalloproteinase concentrations grouped in the normal range, whereas high concentrations of several matrix metalloproteinases are found in some disease state patients. MMP-13 was undetectable in these serum samples. NOTE: MMP levels can vary between plasma and serum samples due to the release of MMPs during serum preparation.



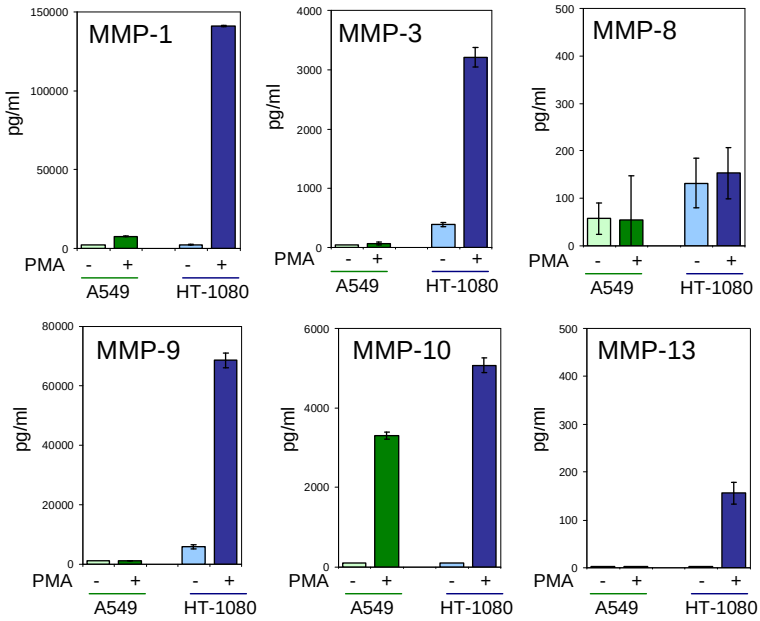
Normal MMP Expression Ranges in Human Serum (n = 14)

	MMP-1	MMP-3	MMP-8	MMP-9	MMP-10	MMP-13
pg/ml	0 - 1300	15000 - 73000	600 – 4000	4000 - 42000	150 - 3800	0 – 21



MMP Secretion by Human Cell Lines

The WideScreen™ Human MMP Panel was used to quantify MMP levels secreted into tissue culture supernatant by human carcinoma cell lines. Cells were serum starved for 24 hours and indicated wells were stimulated with PMA (50 ng/ml, Calbiochem Cat. No. 524400) for an additional 24 hours. Supernatants were harvested, clarified, and diluted 5-fold prior to testing. All targets were undetectable in tissue culture media alone.



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Performance Characteristics:

Note: Representative data are shown (not Lot-specific).

Sensitivity:

The measured Lower Limits of Detection (LLOD) of the Human MMP Panel are shown in pg/ml. The LLOD were determined by adding two standard deviations to the average MFI value of twenty replicate blank wells. The corresponding concentrations (pg/ml) were obtained by interpolation from the standard curves.

Assay	LLOD (pg/ml)
MMP-1	24
MMP-3	4
MMP-8	28
MMP-9	10
MMP-10	3
MMP-13	22

Recovery and Linearity:

To assess analyte recovery from serum, a mid-range concentration of purified standards was spiked into 14 different human serum samples. Measurements from the spiked samples (minus the level in unspiked samples) were compared to the expected values.

Linearity was measured by serially diluting serum samples containing physiological levels of MMPs in Diluent B. Back-calculated concentrations at each dilution were compared to the measurement from the 20-fold dilution (defined as 100%). MMP-containing serum samples were either disease-state (MMP-3 and MMP-9) or purified standards spiked into serum.

	Recovery Serum (n = 14)		Linearity (% of expected)		
Assay	**Average**	**Range**	**1/20**	**1/100**	**1/200**
MMP-1	107%	(81-122%)	100%	92%	83%
MMP-3	120%	(37-199%)	100%	113%	104%
MMP-8	89%	(51-130%)	100%	113%	130%
MMP-9	108%	(48-145%)	100%	125%	133%
MMP-10	88%	(34-119%)	100%	138%	144%
MMP-13	139%	(111-165%)	100%	86%	93%

Specificity:

The Human MMP Panel was tested for cross-recognition against the individual MMP standards using the highest concentration of the standard dilution series. Cross-recognition was < 1% for all assays with the exception of the MMP-3 assay, which shows approximately 3% cross-recognition of MMP-10.

This kit recognizes latent (pro), active, and TIMP-bound forms of the human MMPs included in this panel. MMP standard and recombinant TIMP-1, TIMP-2, TIMP-3, or TIMP-4 were complexed in a 1:1 ratio with the MMP Standard premix for 2 hours at room temperature. TIMP interference with MMP quantification was negligible (within error of the assay) unless noted in the table below.

Assay Interference by TIMPs

	MMP-1	MMP-3	MMP-8	MMP-9	MMP-10	MMP-13
TIMP-1	–	–	–	–	–	–
TIMP-2	–	–	–	–	–	–
TIMP-3	–	–	–	6 %	–	6 %
TIMP-4	–	–	–	–	–	–

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Precision:

Reproducibility within an assay (intra-assay precision) was tested using two samples of known concentrations 16 times on the same plate. For the Human MMP Panel, the MFI values of these measurements had a percent Coefficient of Variation (% CV) range of 4–11%. Reproducibility between assays (inter-assay precision) was tested using two samples of known concentrations, in three separate experiments. For the Human MMP Panel, the quantitative values (pg/ml) of these measurements had a percent Coefficient of Variation (% CV) range of 2–10%.

Intra-assay Precision

Assay	Sample	N =	MFI	SD	% CV
MMP-1	1	16	8214	876	11
	2	16	3605	228	6
MMP-3	1	16	21503	1402	7
	2	16	13305	794	6
MMP-8	1	16	3536	205	6
	2	16	1664	134	8
MMP-9	1	16	14939	690	5
	2	16	8233	302	4
MMP-10	1	16	22920	1005	4
	2	16	13831	574	4
MMP-13	1	16	20512	1181	6
	2	16	8999	409	5

Inter-assay Precision

Assay	Sample	Exp. 1 (pg/ml)	Exp. 2 (pg/ml)	Exp. 3 (pg/ml)	Total % CV
MMP-1	1	528	639	547	10
	2	200	198	167	10
MMP-3	1	667	603	571	8
	2	214	197	212	5
MMP-8	1	3480	3930	3663	6
	2	1267	1270	1140	6
MMP-9	1	585	619	559	5
	2	211	214	222	3
MMP-10	1	595	637	578	5
	2	211	202	209	2
MMP-13	1	568	641	540	9
	2	174	180	209	10

Product: Widescreen™ Human MMP Panel

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Lot Specific Quality Control Assays:

Test Conditions: All components of the WideScreen™ Human MMP Panel have been verified to meet quality specifications of consistent lot-to-lot performance in quantitative immunoassays for the xMAP® platform.

Certified By: K. Stephens
For Research Use Only.

Warranty: EMD Chemicals Inc. warrants that the product will meet or exceed these specifications when used (under normal conditions) in your laboratory. We will promptly replace the product free of charge if the product does not conform to these specifications. Our obligation and your sole remedy is limited to such replacement in the event that the product proves to be defective. No other warranties are expressed or implied, including, but not limited to, the implied warranties of merchantability and fitness for a particular purpose. EMD Chemicals Inc. is not liable for consequential damages.

By opening the packaging containing this product (which contains fluorescently labeled microsphere beads authorized by Luminex Corporation) or using this product in any manner, you, the Buyer are consenting and agreeing to be bound by the following terms and conditions. You are also agreeing that the following terms and conditions constitute a legally valid and binding contract that is enforceable against you. If you do not agree to all of the terms and conditions set forth below, you must promptly return this product for a full refund prior to using it in any manner.

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THIS PRODUCT IS FOR RESEARCH USE ONLY.