PRODUCT INFORMATION & MANUAL

Human MMP3 Platinum ELISA

BMS2014/2 / BMS2014/2TEN

Enzyme-linked Immunosorbent Assay for quantitative detection of human MMP3.

For research use only.
Not for diagnostic or therapeutic procedures.

eBioscience®
An Affymetrix Company

Human MMP3 Platinum ELISA

North America

Technical Support:

Research Products:
888.810.6168
858.642.2058
tech@eBioscience.com

Clinical Products:
877.726.8559
858.642.2058
tech@eBioscience.com

Customer Service:
888.999.1371
858.642.2058
info@eBioscience.com

Fax:
858.642.2046

Europe/International*

Technical Support:
+43 1 796 40 40-120
tech@eBioscience.com

Customer Service:
+43 1 796 40 40-304
info@eBioscience.com

Fax:
+43 1 796 40 40-400

Bender MedSystems GmbH
Campus Vienna Biocenter 2
1030 Vienna, Austria
www.eBioscience.com

* Customers outside North America and Europe may contact their eBioscience distributor listed on our website at www.eBioscience.com/distributors.

TABLE OF CONTENTS

1 Intended Use

The human MMP3 ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of human MMP3. **The human MMP3 ELISA is for research use only. Not for diagnostic or therapeutic procedures.**

2 Summary

The human MMP-3 ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of total human MMP-3. The human MMP-3 ELISA is for research use only. Not for diagnostic or therapeutic procedures.

The matrix metalloproteinases (MMPs) represent a family of more than 15 Zn-dependent endopeptidases with extracellular activity. They share a common conserved protease domain sequence in which three His residues form a complex with a catalytic Zn ion. These enzymes are responsible for the degradation and remodelling of extracellular matrix (ECM) components such as collagen, aggrecan, laminin, fibrilin and fibronectin.

MMP-3 also known as, matrix metalloproteinase-3 or stromelysin-1, is secreted as a zymogen by connective tissue cells that requires protelytic cleavage of the attached pro-peptide domain for activation. Increased serum levels of MMP-3 are observed in inflammatory rheumatic diseases. MMP expression also correlates strongly with invasive behavior of tumor cells in culture and with metastasis in animal models. Overexpression of SL-1 can facilitate cell infiltration into surrounding tissue and degrade the ECM leading to induction of apoptosis.

MMP-3 levels are a good marker for several inflammatory disease states.

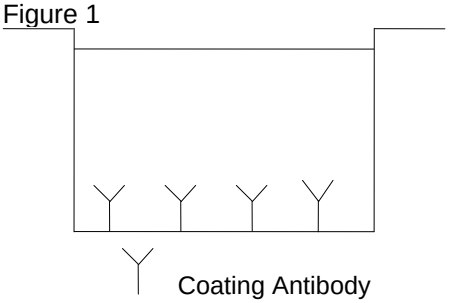
For literature update refer to **www.eBioscience.com**

3 Principles of the Test

An anti-human MMP3 coating antibody is adsorbed onto microwells.

Coated Microwell

Figure 1



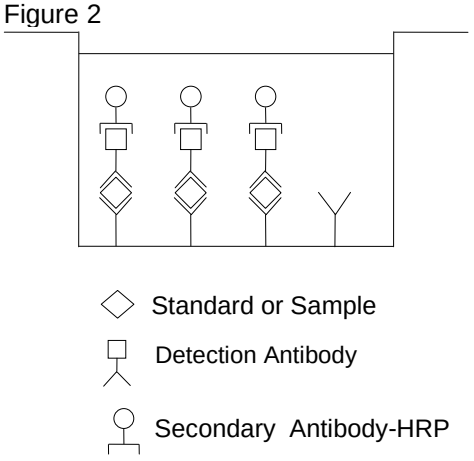
Human MMP3 present in the sample or standard binds to antibodies adsorbed to the microwells. A Detection Antibody Mixture (non-conjugated anti-human MMP3 antibody and a HRP-labelled secondary antibody) is added.

Non-conjugated anti-human MMP3 antibody binds to human MMP3 captured by the first antibody.

Secondary-Antibody-HRP binds to the non-conjugated anti-human MMP3 antibody.

First Incubation

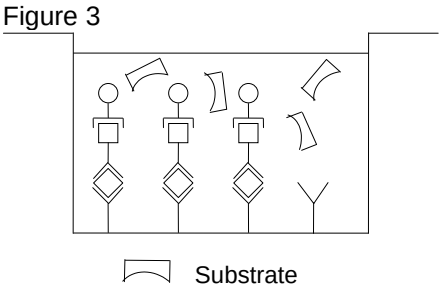
Figure 2



Following incubation unbound Secondary-Antibody -HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

Second Incubation

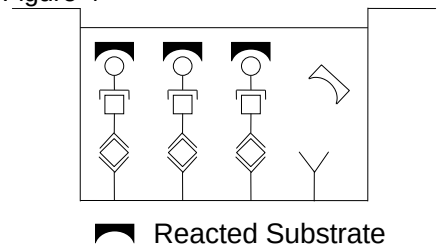
Figure 3



A coloured product is formed in proportion to the amount of human MMP3 present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 human MMP3 standard dilutions and human MMP3 sample concentration determined.

Third Incubation

Figure 4



Reacted Substrate

4 Reagents Provided

4.1 Reagents for human MMP3 ELISA BMS2014/2 (96 tests)

1 aluminium pouch with a **Microwell Plate** coated with monoclonal antibody to human MMP3

1 vial (150 µl) **Detection Antibody Mixture** containing **unlabeled** anti-human MMP3 polyclonal antibody (rabbit) mixed with goat **anti-rabbit-HRP**

2 vials human MMP3 **Standard** lyophilized, 8 ng/mL upon reconstitution

1 vial (5 ml) **Assay Buffer Concentrate** 20x
(PBS with 1% Tween 20 and 10% BSA)

1 bottle (50 ml) **Wash Buffer Concentrate** 20x
(PBS with 1% Tween 20)

1 vial (15 ml) **Substrate Solution** (tetramethyl-benzidine)

1 vial (12 ml) **Stop Solution** (1M Phosphoric acid)

1 vial (0.4 ml) **Blue-Dye**

1 vial (0.4 ml) **Green-Dye**

4 **Adhesive Films**

4.2 Reagents for human MMP3 ELISA BMS2014/2TEN(10x96 tests)

10 aluminium pouches with a **Microwell Plate coated** with monoclonal antibody to human MMP3

10 vials (150 µl) **Detection Antibody Mixture** containing **unlabeled** anti-human MMP3 polyclonal antibody (rabbit) mixed with goat **anti-rabbit-HRP**

10 vials human MMP3 **Standard** lyophilized, 8 ng/mL upon reconstitution*

2 vials (5 ml) **Assay Buffer Concentrate** 20x
(PBS with 1% Tween 20 and 10% BSA)

5 bottles (50 ml) **Wash Buffer Concentrate** 20x
(PBS with 1% Tween 20)

10 vials (15 ml) **Substrate Solution** (tetramethyl-benzidine)

10 vials (12 ml) **Stop Solution** (1M Phosphoric acid)

6 vials (0.4 ml) **Blue-Dye**

6 vials (0.4 ml) **Green-Dye**

20 **Adhesive Films**

5 Storage Instructions – ELISA Kit

Store kit reagents between 2°C and 8°C. Immediately after use remaining reagents should be returned to cold storage (2°C to 8°C). Expiry of the kit and reagents is stated on labels.

Expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

6 Specimen Collection and Storage Instructions

Cell culture supernatant, serum and plasma (citrate, heparin) were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum or plasma from the clot or cells as soon as possible after clotting and separation.

Pay attention to a possible **“Hook Effect”** due to high sample concentrations (see chapter 11)

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive human MMP3. If samples are to be run within 24 hours, they may be stored at 2° to 8°C (for stability refer to 0).
Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

7 Materials Required But Not Provided

- 5 ml and 10 ml graduated pipettes
- 5 µl to 1000 µl adjustable single channel micropipettes with disposable tips
- 50 µl to 300 µl adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microplate shaker
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform regression analysis

8 Precautions for Use

- All reagents should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipette by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or specimens.
- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.

- Exposure to acid inactivates the conjugate.
- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.
- Decontaminate and dispose specimens and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

9 Preparation of Reagents

Buffer Concentrates should be brought to room temperature and should be diluted before starting the test procedure.
If crystals have formed in the **Buffer Concentrates**, warm them gently until they have completely dissolved.

9.1 Wash Buffer (1x)

Pour entire contents (50 ml) of the **Wash Buffer Concentrate** (20x) into a clean 1000 ml graduated cylinder. Bring to final volume of 1000 ml with glass-distilled or deionized water. Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2° to 25°C. Please note that Wash Buffer (1x) is stable for 30 days.

Wash Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (20x) (ml)	Distilled Water (ml)
1 - 6	25	475
1 - 12	50	950

9.2 Assay Buffer (1x)

Pour the entire contents (5 ml) of the **Assay Buffer Concentrate** (20x) into a clean 100 ml graduated cylinder. Bring to final volume of 100 ml with distilled water. Mix gently to avoid foaming.

Store at 2° to 8°C. Please note that the Assay Buffer (1x) is stable for 30 days.

Assay Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (20x) (ml)	Distilled Water (ml)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

9.3 Detection Antibody Mixture

Please note that the Detection Antibody Mixture should be used within 30 minutes after dilution.

The **Detection Antibody Mixture** (unlabeled rabbit-antibody mixed with goat anti-rabbit-HRP) must be diluted 1:100 with Assay Buffer (1x) just prior to use in a clean plastic test tube. Detection Antibody Mixture may be prepared as needed according to the following table:

Number of Strips	Detection Antibody Mixture (ml)	Assay Buffer (1x) (ml)
1 - 6	0.06	5.94
1 - 12	0.12	11.88

9.4 Human MMP3 Standard

Reconstitute **human MMP3 standard** by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 8 ng/mL).

Allow the standard to reconstitute for 10-30 minutes. Mix well prior to making dilutions.

The standard has to be used immediately after reconstitution and cannot be stored.

Standard dilutions can be prepared directly on the microwell plate (see 10.c) or alternatively in tubes (see 9.4.1).

9.4.1 External Standard Dilution

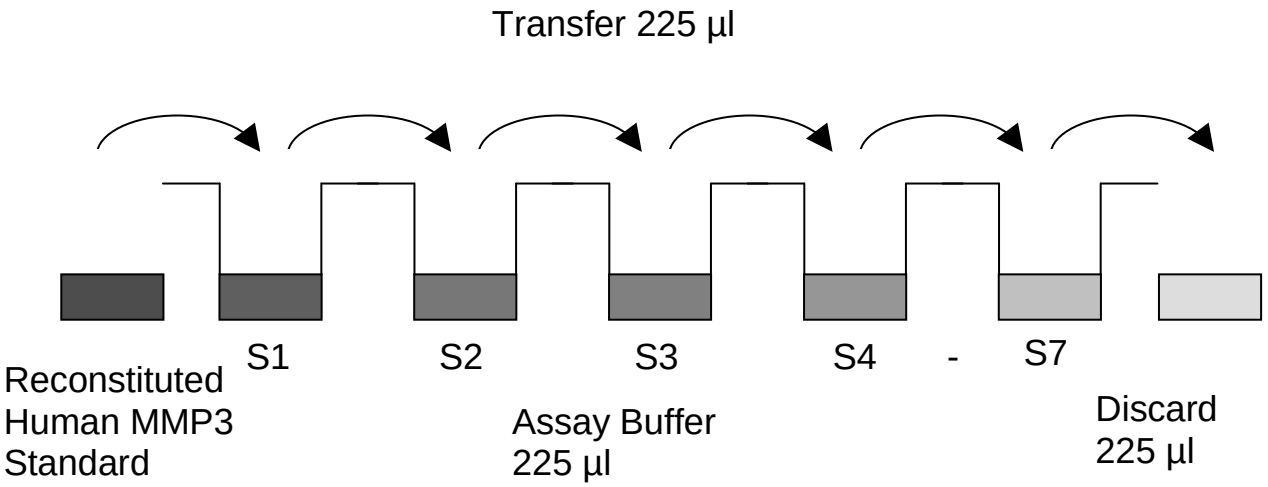
Label 7 tubes, one for each standard point.

S1, S2, S3, S4, S5, S6, S7

Then prepare 2-fold serial dilutions for the standard curve as follows:
Pipette 225 µl of Assay Buffer (1x) into each tube.
Pipette 225 µl of reconstituted standard (concentration = 8 ng/mL) into the first tube, labelled S1, and mix (concentration of S1 = 4 ng/mL).
Pipette 225 µl of this dilution into the second tube, labelled S2, and mix thoroughly before the next transfer.
Repeat serial dilutions 5 more times thus creating the points of the standard curve (see Figure 5).

Assay Buffer (1x) serves as blank.

Figure 5



9.5 Addition of Colour-giving Reagents: Blue-Dye, Green-Dye

In order to help our customers to avoid any mistakes in pipetting the Platinum ELISAs, eBioscience offers a tool that helps to monitor the addition of even very small volumes of a solution to the reaction well by giving distinctive colours to each step of the ELISA procedure.

This procedure is optional, does not in any way interfere with the test results, and is designed to help the customer with the performance of the test, but can also be omitted, just following the instruction booklet.

Alternatively, the dye solutions from the stocks provided (***Blue-Dye, Green-Dye***) can be added to the reagents according to the following guidelines:

1. Diluent:

Before standard and sample dilution add the ***Blue-Dye*** at a dilution of 1:250 (see table below) to the appropriate diluent (1x) according to the test protocol. After addition of ***Blue-Dye***, proceed according to the instruction booklet.

5 ml Assay Buffer	20 µl ***Blue-Dye***
12 ml Assay Buffer	48 µl ***Blue-Dye***
50 ml Assay Buffer	200 µl ***Blue-Dye***

2. Detection Antibody Mixture:

Before dilution of the concentrated Detection Antibody Mixture, add the ***Green-Dye*** at a dilution of 1:100 (see table below) to the Assay Buffer (1x) used for the final dilution. Proceed after addition of ***Green-Dye*** according to the instruction booklet: Preparation of Detection Antibody Mixture.

3 ml Assay Buffer (1x)	30 µl ***Green-Dye***
6 ml Assay Buffer (1x)	60 µl ***Green-Dye***

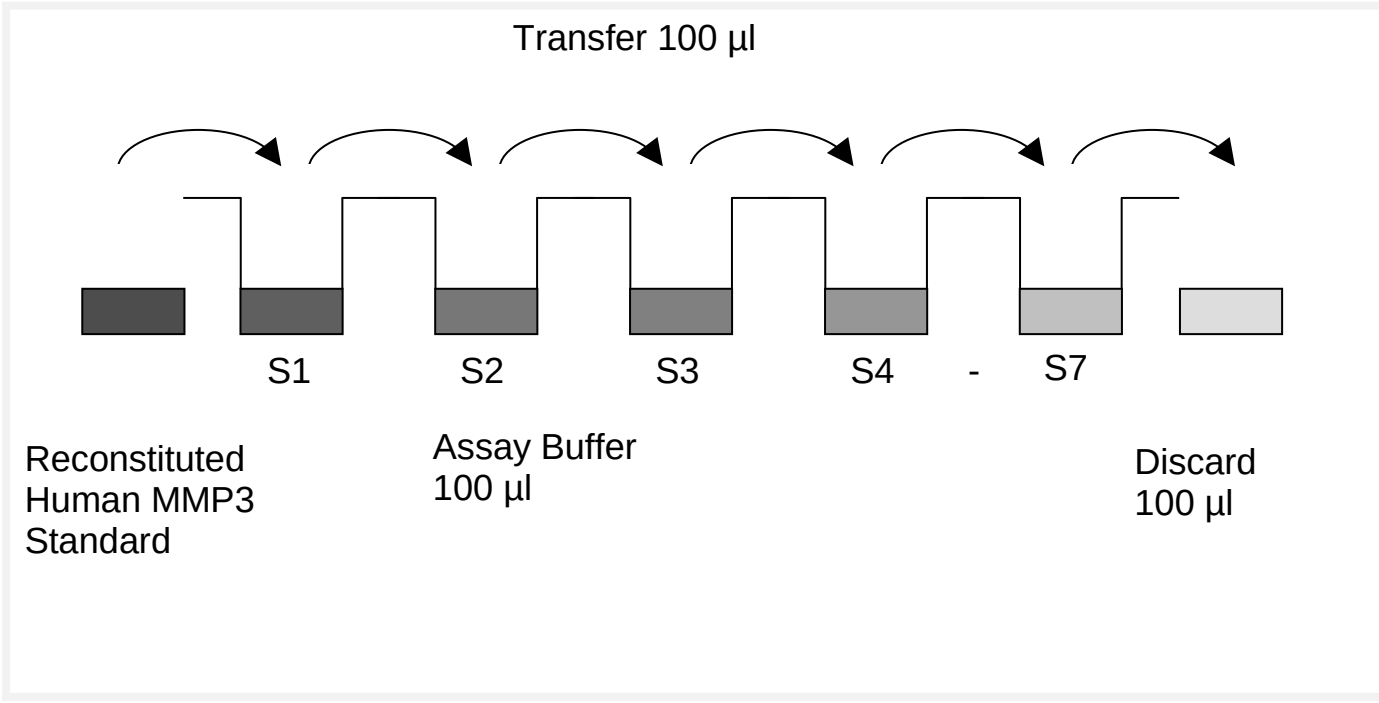
10 Test Protocol

a. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control sample should be assayed in duplicate. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2°-8°C sealed tightly.

b. Wash the microwell strips twice with approximately 400 µl **Wash Buffer** per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about **10 – 15 seconds** before aspiration. Take care not to scratch the surface of the microwells.
After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. **Do not allow wells to dry**.

c. **Standard dilution on the microwell plate** (Alternatively the standard dilution can be prepared in tubes – see 9.4.1):
Add 100 µl of Assay Buffer (1x) in duplicate to all **standard wells**.
Pipette 100 µl of prepared **standard** (see Preparation of Standard 9.4, concentration = 8 ng/mL), in duplicate, into well A1 and A2 (see Table 1). Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1 S1 = 4 ng/mL), and transfer 100 µl to wells B1 and B2, respectively (see Figure 7). Take care not to scratch the inner surface of the microwells. Continue this procedure 5 times, creating two rows of human MMP3 standard dilutions, ranging from 4 ng/mL to 0.06 ng/mL. Discard 100 µl of the contents from the last microwells (S7) used.

Figure 6


Transfer 100 µl
S1
S2
S3
S4
-
S7
Reconstituted Human MMP3 Standard
Assay Buffer 100 µl
Discard 100 µl

In case of an **external standard dilution** (see 9.4.1), pipette 100 µl of these standard dilutions (S1 – S7) in the standard wells according to Table 1.

Table 1

Table depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	**2**	**3**	**4**
A	Standard 1 (4.00 ng/mL)	Standard 1 (4.00 ng/mL)	Sample 1	Sample 1
B	Standard 2 (2.00 ng/mL)	Standard 2 (2.00 ng/mL)	Sample 2	Sample 2
C	Standard 3 (1.00 ng/mL)	Standard 3 (1.00 ng/mL)	Sample 3	Sample 3
D	Standard 4 (0.50 ng/mL)	Standard 4 (0.50 ng/mL)	Sample 4	Sample 4
E	Standard 5 (0.25 ng/mL)	Standard 5 (0.25 ng/mL)	Sample 5	Sample 5
F	Standard 6 (0.13 ng/mL)	Standard 6 (0.13 ng/mL)	Sample 6	Sample 6
G	Standard 7 (0.06 ng/mL)	Standard 7 (0.06 ng/mL)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

d. Add 100 µl of Assay Buffer (1x) in duplicate to the **blank wells**.

e. Add 90 µl of Assay Buffer to the **sample wells**.

f. Add 10 µl of each sample in duplicate to the **sample wells**.

g. Cover with an adhesive film and incubate at room temperature (18 to 25°C) for 2 hours, on a microplate shaker set at 400 rpm. **(Shaking is absolutely necessary for an optimal test performance.)**

h. Prepare **Detection Antibody Mixture** (1:100 in Assay Buffer).

i. Remove adhesive film and empty wells. **Wash** microwell strips 4 times according to point b of the test protocol. Proceed immediately to the next step.

j. Pipette 100 µl of **Detection Antibody Mixture** to all wells.

k. Cover with an adhesive film and incubate at room temperature (18 to 25°C) for 1 hour, on a microplate shaker set at 400 rpm. **(Shaking is absolutely necessary for an optimal test performance.)**

l. Remove adhesive film and empty wells. **Wash** microwell strips 4 times according to point b of the test protocol. Proceed immediately to the next step.

m. Pipette 100 µl of **TMB Substrate Solution** to all wells.

n. Incubate the microwell strips at room temperature (18° to 25°C) for 30 minutes. Avoid direct exposure to intense light.

The colour development on the plate should be monitored and the substrate reaction stopped (see next point of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for colour development has to be done individually for each assay.

It is recommended to add the stop solution when the highest standard has developed a dark blue colour. Alternatively the colour development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 – 0.95.

o. Stop the enzyme reaction by quickly pipetting 100 µl of **Stop Solution** into each well. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 - 8°C in the dark.

p. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the standards.

Shaking is absolutely necessary for an optimal test performance.

11 Calculation of Results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean value.

- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human MMP3 concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).

- To determine the concentration of circulating human MMP3 for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding human MMP3 concentration.

- **If instructions in this protocol have been followed samples have been diluted 1:10 (10 µl sample + 90 µl Assay Buffer), the concentration read from the standard curve must be multiplied by the dilution factor (x 10).**

- **Calculation of samples with a concentration exceeding standard 1 will result in incorrect, low human MMP3 levels (Hook Effect). Such samples require further external predilution according to expected human MMP3 values with Assay Buffer in order to precisely quantitate the actual human MMP3 level.**

- It is suggested that each testing facility establishes a control sample of known human MMP3 concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.

- A representative standard curve is shown in Figure 7. This curve cannot be used to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

Representative standard curve for human MMP3 ELISA. Human MMP3 was diluted in serial 2-fold steps in Assay Buffer (1x). Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

Figure 7

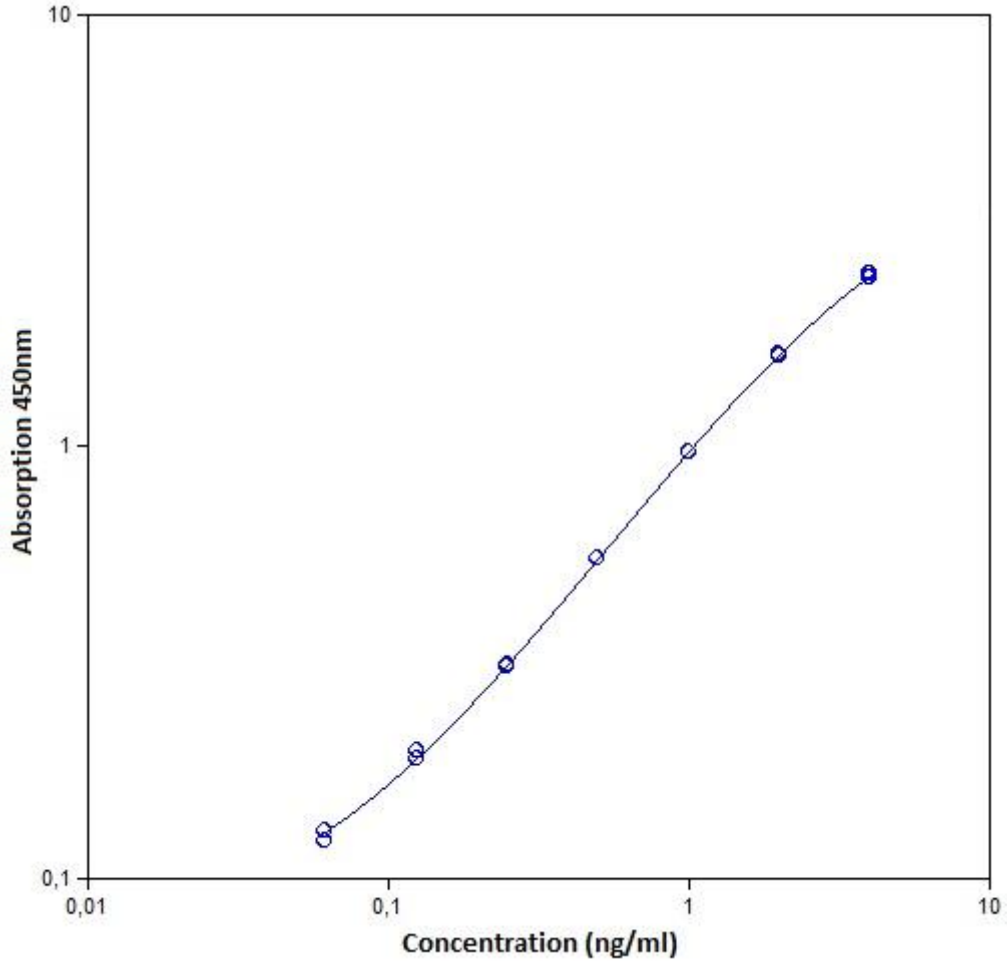


Table 2

Typical data using the human MMP3 ELISA
Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	human MMP3 Concentration (ng/mL)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	4.00	2.428 2.483	2.455	1.1
2	2.00	1.616 1.627	1.622	0.4
3	1.00	0.960 0.960	0.960	0.0
4	0.50	0.546 0.549	0.548	0.3
5	0.25	0.310 0.307	0.308	0.5
6	0.13	0.196 0.187	0.192	2.3
7	0.06	0.121 0.128	0.125	2.9
Blank	0.00	0.063 0.062	0.062	0.7

The OD values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects). Furthermore shelf life of the kit may affect enzymatic activity and thus colour intensity. Values measured are still valid.

12 Limitations

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

13 Performance Characteristics

13.1 Sensitivity

The limit of detection of human MMP3 defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 0.008 ng/mL (mean of 8 independent assays).

13.2 Reproducibility

13.2.1 Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with replicates of 8 serum, plasma (citrat, heparin), cell culture supernatant samples containing different concentrations of human MMP3. 2 standard curves were run on each plate. Data below show the mean human MMP3 concentration and the coefficient of variation for each sample (see Table 3). The calculated overall intra-assay coefficient of variation was 5.8%.

Table 3

The mean human MMP3 concentration and the coefficient of variation for each sample

Sample	Experiment	Mean human MMP3 Concentration (ng/mL)	Coefficient of Variation (%)
1	1	26.3	5.2
	2	24.8	8.5
	3	24.9	4.2
2	1	25.0	7.8
	2	24.0	3.5
	3	23.6	4.8
3	1	15.5	4.7
	2	14.7	3.6
	3	14.8	2.6
4	1	6.2	5.2
	2	5.8	3.0
	3	5.7	13.7
5	1	7.5	6.6
	2	7.4	7.2
	3	7.3	8.1
6	1	19.7	6.8
	2	19.4	5.0
	3	18.7	5.2
7	1	15.6	8.5
	2	15.1	5.0
	3	15.2	3.4
8	1	18.7	6.7
	2	17.6	5.8
	3	17.3	4.3

13.2.2 Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum and plasma samples containing different concentrations of human MMP3. 2 standard curves were run on each plate. Data below show the mean human MMP3 concentration and the coefficient of variation calculated on 18 determinations of each sample (see Table 4). The calculated overall inter-assay coefficient of variation was 3.0%.

Table 4
The mean human MMP3 concentration and the coefficient of variation of each sample

Sample	Mean human MMP3 Concentration (ng/mL)	Coefficient of Variation (%)
1	25.3	3.3
2	24.2	3.0
3	15.0	2.9
4	5.9	4.7
5	7.4	1.7
6	19.3	2.6
7	15.3	1.9
8	17.9	4.1

13.3 Spike Recovery

The spike recovery was evaluated by spiking 3 levels of human MMP3 into serum, plasma (heparin, citrate) and cell culture supernatant. Recoveries were determined with 2 replicates each. The amount of endogenous human MMP3 in unspiked samples was subtracted from the spike values.

For recovery data see Table 5.

Table 5

Sample matrix	Spike high		Spike medium		Spike low	
	Mean (%)	Range (%)	Mean (%)	Range (%)	Mean (%)	Range (%)
Serum	113	102-120	95	86-106	73	60-84
Plasma (citrate)	120	110-130	84	64-97	80	68-94
Plasma (heparin)	118	111-127	107	101-112	75	61-97
Cell culture supernatant	121	121	106	104-108	101	99-104

13.4 Dilution Parallelism

Serum, plasma (citrate, heparin), cell culture supernatant samples with different levels of human MMP3 were analyzed at serial 2 fold dilutions with 4 replicates each.

For data see Table 6.

Table 6

Sample matrix	Recovery of Exp. Val.		
	Dilution	Mean (%)	Range (%)
Serum	1:20	101	95-111
	1:40	109	99-128
	1:80	112	101-123
Plasma (citrate)	1:20	109	103-115
	1:40	117	113-122
	1:80	119	111-122
Plasma (heparin)	1:20	86	79-96
	1:40	86	78-100
	1:80	86	79-99
Cell culture supernatant	1:20	93	87-98
	1:40	89	86-93
	1:80	90	88-92

13.5 Sample Stability

13.5.1 Freeze-Thaw Stability

Aliquots of serum, plasma, cell culture supernatant samples (spiked or unspiked) were stored at -20°C and thawed 3 times, and the human MMP3 levels determined.

There was no significant loss of human MMP3 immunoreactivity detected by freezing and thawing.

13.5.2 Storage Stability

Aliquots of serum, plasma, cell culture supernatant samples (spiked or unspiked) were stored at -20°C, 2-8°C and room temperature (RT), and the human MMP3 level determined after 24 h.

There was no significant loss of human MMP3 immunoreactivity detected during storage under above conditions.

13.6 Specificity

The assay detects both natural and recombinant human MMP3.
The cross reactivity and interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a human MMP3 positive sample.

There was no cross reactivity or interference detected.

13.7 Expected Values

Panels of 40 serum as well as plasma samples (citrate, heparin) were tested for human MMP3.

For detected human MMP3 levels see Table 7.

Table 7

Sample Matrix	Number of Samples Evaluated	Mean ng/mL	Range ng/mL	Standard Deviation ng/mL
Serum	40	14.9	3.9-60	12.8
Plasma (Citrate)	40	13.2	2.2-46.6	9.8
Plasma (Heparin)	40	15.1	5.4-35.6	8.2

14 Ordering Information

North America

Technical Support:

Research Products:
888.810.6168
858.642.2058
tech@eBioscience.com

Clinical Products:
877.726.8559
858.642.2058
tech@eBioscience.com

Customer Service:
888.999.1371
858.642.2058
info@eBioscience.com

Fax:
858.642.2046

Europe/International*

Technical Support:
+43 1 796 40 40-120
tech@eBioscience.com

Customer Service:
+43 1 796 40 40-304
info@eBioscience.com

Fax:
+43 1 796 40 40-400

Bender MedSystems GmbH
Campus Vienna Biocenter 2
1030 Vienna, Austria
www.eBioscience.com

* Customers outside North America and Europe may contact their eBioscience distributor listed on our website at www.eBioscience.com/distributors.

15 Reagent Preparation Summary

15.1 Wash Buffer (1x)

Add **Wash Buffer Concentrate** 20x (50 ml) to 950 ml distilled water.

Number of Strips	Wash Buffer Concentrate (ml)	Distilled Water (ml)
1 - 6	25	475
1 - 12	50	950

15.2 Assay Buffer (1x)

Add **Assay Buffer Concentrate** 20x (5 ml) to 95 ml distilled water.

Number of Strips	Assay Buffer Concentrate (ml)	Distilled Water (ml)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

15.3 Detection Antibody Mixture

Make a 1:100 dilution of **Detection Antibody Mixture** in Assay Buffer (1x):

Number of Strips	Detection Antibody Mixture (ml)	Assay Buffer (1x) (ml)
1 - 6	0.06	5.94
1 - 12	0.12	11.88

15.4 Human MMP3 Standard

Reconstitute **human MMP3 standard** with distilled water.
(Reconstitution volume is stated on the label of the standard vial.)

16 Test Protocol Summary

1. Determine the number of microwell strips required.
2. Wash microwell strips twice with Wash Buffer.
3. Standard dilution on the microwell plate: Add 100 µl Assay Buffer, in duplicate, to all standard wells. Pipette 100 µl prepared standard into the first wells and create standard dilutions by transferring 100 µl from well to well. Discard 100 µl from the last wells.
4. Add 100 µl Assay Buffer, in duplicate, to the blank wells.
5. Add 90 µl Assay Buffer to sample wells.
6. Add 10 µl sample in duplicate, to designated sample wells.
7. Cover microwell strips and incubate 2 hours at room temperature (18° to 25°C) on a microplate shaker.
8. Prepare Detection Antibody Mixture (1:100 in Assay Buffer).
9. Empty and wash microwell strips 4 times with Wash Buffer.
10. Add 100 µl diluted Detection Antibody Mixture to all wells.
11. Cover microwell strips and incubate 1 hour at room temperature (18º to 25ºC) on a microplate shaker.
12. Empty and wash microwell strips 4 times with Wash Buffer.
13. Add 100 µl of TMB Substrate Solution to all wells.
14. Incubate the microwell strips for 30 minutes at room temperature (18°to 25°C).
15. Add 100 µl Stop Solution to all wells.
16. Blank microwell reader and measure colour intensity at 450 nm.

If instructions in this protocol have been followed samples have been diluted 1:10 (10 µl sample + 90 µl Assay Buffer), the concentration read from the standard curve must be multiplied by the dilution factor (x 10).

Shaking is absolutely necessary for an optimal test performance.