DRG® MMP-13 (human) Elisa (EIA-4862)

As of 23 Dec. 2008 (Vers. 1.0)

RUO

INTENDED USE

The MMP-13 ELISA is an enzyme-linked immunosorbent assay for quantitative detection of human MMP-13 in cell culture supernatants, human serum, plasma or other body fluids.

The MMP-13 ELISA is for research use only. Not for use in diagnostic or therapeutic procedures.

SUMMARY

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. Further, all MMPs contain a regulatory domain with a conserved motif.

The family of MMPs are capable of breaking down any extracellular matrix component. In normal physiology, MMPs produced by connective tissue are thought to contribute to tissue remodeling in development, in the menstrual cycle and in repair processes following tissue damage. In pathological situations mainly the involvement of breakdown of the connective tissues like in rheumatoid arthritis, cancer and periodontal and cardiovascular diseases are described. Leukocytes, mainly macrophages, are the major sources of MMP production which allow leukocytes to extravasate and penetrate tissues, which is a key event in inflammatory diseases. In parallel, metastatic cancer cells use MMPs to migrate to foreign tissue.

MMP-13, also known as Collagenase-3, is a member of the matrix metralloproteinases family with broad substrate specificity and a potential role in tumor metastasis and invasion (1). The three-dimensional structure reveals a core domain for the protein consisting of three alpha-helices and five beta-sheet strands with an overall tertiary fold similar to the catalytic domain of the other matrix metalloproteinase family members (2). The purified monomeric enzyme has a molecular mass of 19,600 (3).

MMP-13 has originally been identified in breast carcinomas. Further studies revealed that this enzyme is produced by a variety of malignant tumors including head and neck squamous cell carcinomas (4, 5, 12), where elevated expression reflects increased tumor invasiveness, squamous cell carcinoma of the upper aerodigestive tract (8), laryngeal squamous cell carcinomas (6) and vulvar squamous cell carcinomas (7).

Recent studies showed the role of MMP-13 as diagnostic marker of prostate cancer (9, 13), a target for breast cancer xenograft monitoring (10). Increased expression is related to cancer aggressiveness in oesophageal cancer (11).

MMP-13, together with other MMPs, is involved in the gingival extracellular matrix degradation during periodontitis (14). The activity of MMP-13 is associated with poorer survival in colorectal cancer (15, 16).

Endothelial cells in the skin have further been shown to be a source of MMP-13 and the expression of the enzyme is upregulated under conditions that promote endothelial cell growth and vascular differentiation (17). The over-expression of MMP-13 associated with nonhealing wounds is shown for chronic dermal ulcers (18).

There is growing body of evidence that MMP-13 is a major player in the disease condition of rheumatoid arthritis (19) and osteoarthritis (20, 21, 22, 23).

PRINCIPLES OF THE TEST

An anti-MMP-13 monoclonal coating antibody is adsorbed onto microwells.

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MMP-13 present in the sample or standard binds to antibodies adsorbed to the microwells; a biotin-conjugated polyclonal anti-MMP-13 antibody is added and binds to MMP-13 captured by the first antibody.

Following incubation unbound biotin conjugated anti-MMP-13 is removed during a wash step. Streptavidin-HRP is added and binds to the biotin conjugated anti-MMP-13. Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

A coloured product is formed in proportion to the amount of MMP-13 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from seven MMP-13 standard dilutions and MMP-13 sample concentration determined.

REAGENTS PROVIDED

1	aluminium pouch with a **Microwell Plate coated** with Monoclonal Antibody (murine) to human MMP-13
1	vial (70 µL) **Biotin-Conjugate** anti-MMP-13 polyclonal antibody (rabbit)
1	vial (150 µL) **Streptavidin-HRP**
2	vials MMP-13 **Standard**, lyophilized, 40 ng/mL upon reconstitution
1	bottle (50 mL) **Wash Buffer** Concentrate 20x (PBS with 1% Tween 20)
1	vial (5 mL) **Assay Buffer** Concentrate 20x (PBS with 1% Tween 20 and 10 % BSA)
1	vial (12 mL) **Sample Diluent**
1	vial (7 mL) **Substrate Solution I** (tetramethyl-benzidine)
1	vial (7 mL) **Substrate Solution II** (0.02 % buffered hydrogen peroxide)
1	vial (12 mL) **Stop Solution** (1M Phosphoric acid)
1	vial (0.4 mL each) **Blue-Dye, Green-Dye, Red-Dye**
4	adhesive **Plate Covers**
	Reagent Labels

STORAGE INSTRUCTIONS

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.

The 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, the reagent is not contaminated by the first handling.

SPECIMEN COLLECTION

Cell culture supernatants, human serum, plasma or other biological samples will be suitable for use in the assay.

Remove serum from the clot or red cells, respectively, as soon as possible after clotting and separation.

Samples containing a visible precipitate must be clarified prior to use in the assay.

Do not use grossly hemolyzed or lipemic specimens.

Clinical samples should be kept at 2°C to 8°C and separated rapidly before storing at -20°C to avoid loss of bioactive MMP-13.

If samples are to be run within 24 hours, they may be stored at 2°C to 8°C. Avoid repeated freeze-thaw cycles.

For stability and suitability of samples refer to respective chapter.

MATERIALS REQUIRED BUT NOT PROVIDED

- 5 mL and 10 mL graduated pipettes
- 10 µL to 1,000 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)
- 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 linear regression analysis.

PRECAUTIONS FOR USE

- All chemicals 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 statements(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 solutions 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 reagents.
- Exposure to acids will inactivate the conjugate.

- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solutions must be at room temperature prior to use.
- Decontaminate and dispose specimens and all potentially contaminated materials as if 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.

PREPARATION OF REAGENTS

1.1 Wash Buffer

If crystals have formed in the Wash Buffer Concentrate, warm it gently until they have completely dissolved.

Pour entire contents (50 mL) of the **Wash Buffer Concentrate** into a clean 1000 mL graduated cylinder. Bring final volume to 1000 mL with glass-distilled or deionized water. Mix gently to avoid foaming. The pH of the final solution should adjust to 7.4.

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

Wash Buffer may be prepared as needed according to the following table:

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

1.2 Assay Buffer

Mix the contents of the bottle well. Add contents of **Assay Buffer** Concentrate (5.0 mL) to 95 mL distilled or deionized water and mix gently to avoid foaming.

Store at 2º to 8ºC. Please note that the Assay Buffer is stable for 30 days.

Assay Buffer may be prepared as needed according to the following table:

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

1.3 Preparation of Biotin-Conjugate

Make a 1:100 dilution of the concentrated **Biotin-Conjugate** solution with **Assay Buffer** (reagent B) in a clean plastic tube as needed according to the following table:

Number of Strips	Biotin-Conjugate(mL)	Assay Buffer(mL)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

1.4 Preparation of MMP-13 Standard

Reconstitute MMP-13 **Standard** by addition of distilled water. Reconstitution volume is stated on the label. Mix gently to ensure complete solubilization.

1.5 Preparation of Streptavidin-HRP

Make a 1:100 dilution of the concentrated **Streptavidin-HRP** solution in a clean plastic tube with **Assay Buffer** as needed according to the following table:

Number of Strips	Streptavidin- HRP(mL)	Assay Buffer(mL)
1 - 6	0.06	5.94
1 - 12	0.12	11.88

1.6 TMB Substrate Solution

Using clean pipettes and containers known to be metal free, dispense an equal volume of **Substrate Solution I** into **Substrate Solution II** and swirl gently to mix. The TMB Substrate Solution may develop a yellow tinge over time. This does not seem to affect product performance. A blue colour present in the TMB Substrate Solution, however, indicates that it has been contaminated and must be discarded. The TMB Substrate Solution must be used within a few minutes after mixing. Warm to room temperature before use. Avoid direct exposure of TMB reagents to intense light and oxidizing agents during storage or incubation.

Substrate preparation according to assay size:

Number of Strips	Substrate Solution I (mL)	Substrate Solution II (mL)
1 - 6	3.0	3.0
1 - 12	6.0	6.0

1.7 Addition of colour-giving reagents: Blue-Dye, Green-Dye, Red-Dye

In order to help our customers to avoid any mistakes in pipetting the ELISAs, a new tool is offered 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, Red-Dye***) can be added to the reagents according to the following guidelines:

1. Diluent: Before 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 Diluent	20 µL ***Blue-Dye***
12 mL Diluent	48 µL ***Blue-Dye***

2. Biotin-Conjugate: Before dilution of the concentrated conjugate, add the ***Green-Dye*** at a dilution of 1:100 (see table below) to the Assay Buffer used for the final conjugate dilution. Proceed after addition of ***Green-Dye*** according to the instruction booklet, preparation of Biotin-conjugate.

3 mL Assay Buffer	30 µL ***Green-Dye***
6 mL Assay Buffer	60 µL ***Green-Dye***
12 mL Assay Buffer	120 µL ***Green-Dye***

3. Streptavidin-HRP: Before dilution of the concentrated Streptavidin-HRP; add the ***Red-Dye*** at a dilution of 1:250 (see table below) to the Dilution Buffer used for the final Streptavidin-HRP dilution. Proceed after addition of ***Red-Dye*** according to the instruction booklet, preparation of Streptavidin-HRP.

6 mL Assay Buffer	24 µL ***Red-Dye***
12 mL Assay Buffer	48 µL ***Red-Dye***

TEST PROTOCOL

a. Mix all reagents thoroughly without foaming before use.

b. 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 coated with Monoclonal Antibody** to human MMP-13 from holder and store in foil bag with the desiccant provided at 2°C-8°C sealed tightly.

c. Wash the microwell strips twice with approximately 300 µL **Wash Buffer** per well with thorough aspiration of microwell contents between washes. Take care not to scratch the surface of the microwells.

After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing or place upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

d. Add 100 µL of **Sample Diluent** in duplicate to all standard wells.
Prepare standard dilutions by pipetting 100 µL of reconstituted (refer to preparation of reagents, 9.4.) **MMP-13 Standard**, in duplicate, into wells A1 and A2.
Mix the contents of wells A1 and A2 by repeated aspiration and ejection, and transfer 100 µL to well B1 and B2, respectively. Take care not to scratch the inner surface of the microwells.
Continue this procedure five times, creating two rows of MMP-13 standard dilutions ranging from 20 to 0.31 ng/mL.
Discard 100 µL of the contents from the last microwells (G1, G2) used.

Figure 1. Preparation of MMP-13 standard dilutions:

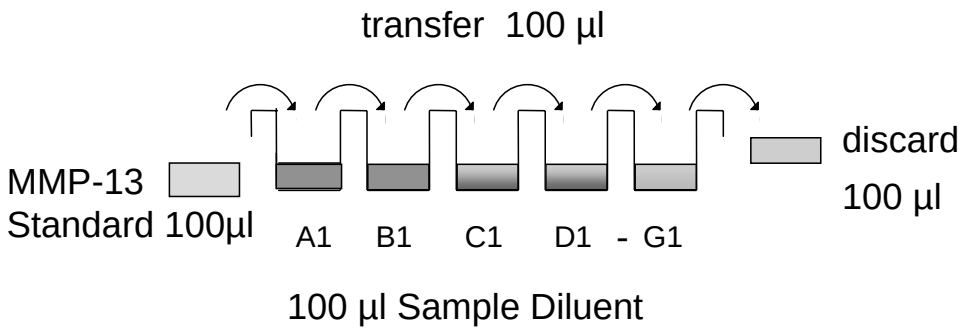

Figure 2. Diagram depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	2	3	4
A	Standard 1 (20 ng/mL)	Standard 1 (20 ng/mL)	Sample 1	Sample 1
B	Standard 2 (10 ng/mL)	Standard 2 (10 ng/mL)	Sample 2	Sample 2
C	Standard 3 (5 ng/mL)	Standard 3 (5 ng/mL)	Sample 3	Sample 3
D	Standard 4 (2.5 ng/mL)	Standard 4 (2.5 ng/mL)	Sample 4	Sample 4
E	Standard 5 (1.25 ng/mL)	Standard 5 (1.25 ng/mL)	Sample 5	Sample 5
F	Standard 6 (0.63 ng/mL)	Standard 6 (0.63 ng/mL)	Sample 6	Sample 6
G	Standard 7 (0.31 ng/mL)	Standard 7 (0.31 ng/mL)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

e. Add 100 µL of **Sample Diluent** in duplicate to the blank wells.

f. Add 50 µL of **Sample Diluent**, in duplicate, to the sample wells.

g. Add 50 µL of each **Sample**, in duplicate, to the designated wells.

h. Prepare **Biotin-Conjugate** (refer to preparation of reagents, 9.3).

i. Add 50 µL of diluted **Biotin-Conjugate** to all wells, including the blank wells.

j. Cover with a **Plate Cover** and incubate at room temperature **(18°C to 25°C)** for 2 hours, if available on a microplate shaker set at 100 rpm.

k. Remove Plate Cover and empty wells. Wash microwell strips 3 times according to point c. of the test protocol. Proceed immediately to the next step.

l. Prepare **Streptavidin-HRP** (refer to preparation of reagents 9.5).

m. Add 100 µL of diluted **Streptavidin-HRP** to all wells, including the blank wells.

n. Cover with a **Plate Cover** and incubate at room temperature **(18°C to 25°C)** for 1 hour, if available on a microplate shaker set at 100 rpm.

o. Prepare **TMB Substrate Solution** a few minutes prior to use (refer to preparation of reagents 9.6).

p. Remove Plate Cover and empty wells. Wash microwell strips 3 times according to point c. of the test protocol. Proceed immediately to the next step.

q. Pipette 100 µL of mixed **TMB Substrate Solution** to all wells, including the blank wells.

r. Incubate the microwell strips at room temperature **(18°C to 25°C)** for about **20 minutes,** if available on a microplate shaker set at 100 rpm. Avoid direct exposure to intense light.
The colour development on the plate should be monitored and the substrate reaction stopped (see point s. of this protocol) before positive wells are no longer properly recordable.
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 an OD of 0.6 – 0.65 is reached.

s. Stop the enzyme reaction by quickly pipetting 100 µL of **Stop Solution** into each well, including the blank wells. 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°C - 8°C in the dark.

t. Read absorbance of each microwell on a spectrophotometer 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 MMP-13 standards.

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

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.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the MMP-13 concentration on the abscissa. Draw a best fit curve through the points of the graph.
- To determine the concentration of circulating MMP-13 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 MMP-13 concentration.
- **For samples which have been diluted according to the instructions given in this manual 1:2, the concentration read from the standard curve must be multiplied by the dilution factor (x2).**
  Note:
  Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low MMP-13 levels. Such samples should be re-analyzed at higher dilution rate in order to precisely quantitate the actual MMP-13 level.
- It is suggested that each testing facility establishes a control sample of known MMP-13 concentration and runs this additional control with each assay. If the values obtained are not within the expected range of this control, the assay results may be invalid.
- A representative standard curve is shown in Figure 3. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of microwell strips assayed.

Figure 3. Representative standard curve for MMP-13 ELISA.
MMP-13 was diluted in serial two-fold steps in Sample Diluent, symbols represent the mean of three parallel titrations. Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

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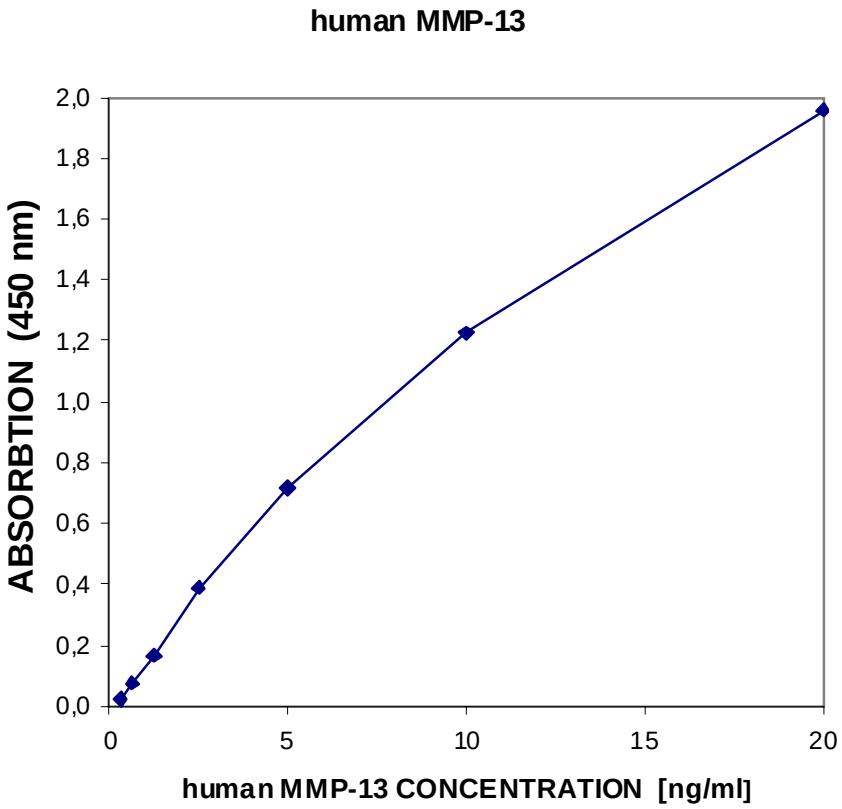

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Typical data using the MMP-13 ELISA
Measuring wavelength: 450 nm, Reference wavelength: 620 nm

Standard	MMP-13 Concentration (ng/mL)	O.D. (450 nm)	O.D. Mean	CV %
1	20	1.985	2.006	1.4
	20	2.026		
2	10	1.273	1.336	6.7
	10	1.399		
3	5	0.733	0.761	5.4
	5	0.788		
4	2.5	0.414	0.420	1.9
	2.5	0.425		
5	1.25	0.181	0.181	0.4
	1.25	0.180		
6	0.63	0.099	0.097	3.7
	0.63	0.094		
7	0.31	0.053	0.054	2.6
	0.31	0.055		
Blank	0	0.033	0.031	9.1
	0	0.029		

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 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. Completely empty wells before dispensing fresh Wash Buffer, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

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PERFORMANCE CHARACTERISTICS

1.8 Sensitivity

The limit of detection of MMP-13 defined as the analyte concentration resulting in an absorption significantly higher than that of the dilution medium (mean plus two standard deviations) was determined to be less than 0.18 ng/mL (mean of 6 independent assays).

1.9 Reproducibility

1.9.1 Intra-assay

Reproducibility within the assay was evaluated in three independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of MMP-13. Two standard curves were run on each plate. Data below show the mean MMP-13 concentration and the coefficient of variation for each sample. The overall intra-assay coefficient of variation has been calculated to be 7.4%.

Positive Sample	Experiment	MMP-13 Concentration (ng/mL)	Coefficient of Variation (%)
1	1	35.2	7
	2	28.4	9
	3	36.0	6
2	1	16.0	7
	2	14.8	10
	3	17.6	6
3	1	6.9	6
	2	7.9	9
	3	8.0	5
4	1	3.0	10
	2	3.4	10
	3	3.2	10
5	1	32.3	7
	2	32.6	6
	3	27.8	4
6	1	12.4	10
	2	12.4	7
	3	13.1	8
7	1	3.1	7
	2	3.9	7
	3	3.7	5
8	1	1.4	6
	2	1.6	8
	3	1.6	9

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1.9.2 Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in three independent experiments by three technicians. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of MMP-13. Two standard curves were run on each plate. Data below show the mean MMP-13 concentration and the coefficient of variation calculated on 18 determinations of each sample. The overall inter-assay coefficient of variation has been calculated to be 8.2%.

Sample	MMP-13 Concentration (ng/mL)	Coefficient of Variation (%)
1	33.2	12.6
2	16.1	8.8
3	7.6	8.5
4	3.2	5.3
5	30.9	8.5
6	12.6	3.2
7	3.5	11.3
8	1.5	7.1

1.10 Spike Recovery

The spike recovery was evaluated by spiking four levels of MMP-13 into 4 different normal human sera. The amount of endogenous MMP-13 in unspiked serum was substracted from the spike values. Mean recovery was 82%.

1.11 Dilution Parallelism

Four serum samples with different levels of MMP-13 were assayed at four serial two-fold dilutions with 4 replicates each. Recoveries ranged from 90.5% to 118.8% with an overall mean recovery of 105.2 %.

Sample	Dilution	MMP-13 Concentration (ng/mL) Expected Value	Observed Value	% Recovery of Exp. Value
1	1:2	--	11.95	--
	1:4	5.98	6.48	108.4 %
	1:8	2.99	3.52	117.6 %
	1:16	1.49	1.78	118.8 %
2	1:2	--	27.60	--
	1:4	13.80	14.58	105.7 %
	1:8	6.90	7.06	102.3 %
	1:16	3.45	3.13	90.7 %
3	1:2	--	6.24	--
	1:4	13.80	14.58	105.7 %
	1:8	6.90	7.06	102.3 %
	1:16	3.45	3.13	90.7 %
4	1:2	--	25.58	--
	1:4	12.79	11.57	90.5 %
	1:8	6.39	6.59	103.0 %
	1:16	3.20	3.17	99.3 %

1.12 Sample Stability

1.12.1 Freeze-Thaw Stability

Aliquots of serum samples were stored frozen at -20°C and thawed up to 5 times, and MMP-13 levels determined. There was no significant loss of MMP-13 by freezing and thawing up to 5 cycles of freezing and thawing.

1.12.2 Storage Stability

Aliquots of serum samples were stored at -20°C, 2-8°C, room temperature (RT), and 37°C and the MMP-13 level determined after 24 h. There was no significant loss of MMP-13 immunoreactivity during above storage conditions.

1.13 Specificity

The interference of circulating factors of the immune systems was evaluated by spiking these proteins at physiologically relevant concentrations into a MMP-13 positive serum. There was no detectable cross reactivity.

1.14 Expected Serum Values

A panel of 40 sera was tested for MMP-13. The detected MMP-13 levels ranged between n.d. and 9.7 ng/mL.

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PREPARATION SUMMARY

A. Wash Buffer

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

B. Assay Buffer

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

C. Biotin-Conjugate

Make a 1:100 dilution according to the table.

Number of Strips	Biotin-Conjugate (mL)	Assay Buffer (mL)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

D. Standard

Add distilled water to each vial of lyophilized **MMP-13 Standard** (volume is stated on the label) as needed.

E. Streptavidin-HRP

Make a 1:100 dilution according to the table.

Number of Strips	Streptavidin-HRP (mL)	Assay Buffer (mL)
1 - 6	0.06	5.94
1 - 12	0.12	11.88

F. TMB Substrate Solution

Number of Strips	Substrate Solution I (mL)	Substrate Solution II (mL)
1 - 6	3.0	3.0
1 - 12	6.0	6.0

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TEST PROTOCOL SUMMARY

- Wash microwell strips twice with **Wash Buffer**
- Add 100 µL **Sample Diluent**, in duplicate, to all standard wells
- Pipette 100 µL reconstituted **MMP-13 Standard** into the first wells and create standard dilutions ranging from 20 to 0.31 ng/mL by trans-ferring 100 µL from well to well. Discard 100 µL from the last wells
- Add 100 µL **Sample Diluent**, in duplicate, to the blank wells
- Add 50 µL **Sample Diluent** to the sample wells
- Add 50 µL **Sample**, in duplicate, to designated wells
- Prepare **Biotin-Conjugate**
- Add 50 µL of diluted **Biotin-Conjugate** to all wells
- Cover microwell strips and incubate 2 hours at room temperature **(18°C to 25°C)** on microplate shaker
- Prepare **Streptavidin-HRP**
- Empty and wash microwell strips 3 times with **Wash Buffer**
- Add 100 µL of diluted **Streptavidin-HRP** to all wells
- Cover microwell strips and incubate 1 hour at room temperature **(18°C to 25°C)** on a microplate shaker
- Prepare **TMB Substrate Solution** few minutes prior to use
- Empty and wash microwell strips 3 times with **Wash Buffer**
- Add 100 µL of mixed **TMB Substrate Solution** to all wells including blank wells
- Incubate the microwell strips for about 20 minutes at room temperature **(18°Cto 25°C)** on a microplate shaker
- Add 100 µL **Stop Solution** to all wells including blank wells
- Blank microwell reader and measure colour intensity at 450 nm

Note: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low MMP-13 levels. Such samples require further dilution with Sample Diluent in order to precisely quantitate the actual MMP-13 level.

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SYMBOLS USED WITH DRG ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..

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