HUMAN MATRIX METALLOPROTEINASE-8 ELISA

Product Data Sheet

Cat. No.: RD194375200R

For Research Use Only
## CONTENTS

1. INTENDED USE 3
2. STORAGE, EXPIRATION 3
3. INTRODUCTION 4
4. TEST PRINCIPLE 6
5. PRECAUTIONS 7
6. TECHNICAL HINTS 7
7. REAGENT SUPPLIED 8
8. MATERIAL REQUIRED BUT NOT SUPPLIED 8
9. PREPARATION OF REAGENTS 9
10. PREPARATION OF SAMPLES 11
11. ASSAY PROCEDURE 12
12. CALCULATIONS 14
13. PERFORMANCE CHARACTERISTICS 15
14. DEFINITION OF THE STANDARD 18
15. PRELIMINARY POPULATION DATA 18
16. TROUBLESHOOTING AND FAQS 19
17. REFERENCES 20
18. EXPLANATION OF SYMBOLS 22

This kit is manufactured by:
BioVendor – Laboratorní medicína a.s.

Use only the current version of Product Data Sheet enclosed with the kit!
1. **INTENDED USE**

The RD194375200R Human Matrix Metalloproteinase-8 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human matrix metalloproteinase-8 (MMP-8).

**Features**

- **It is intended for research use only**
- The total assay time is less than 3 hours
- Serum, plasma (ETDA, citrate, heparin) and saliva samples were tested with the kit – please refer to chapter 10, 13
- Assay format is 96 wells
- Standard is recombinant protein based
- Components of the kit are provided ready to use, concentrated or lyophilized

2. **STORAGE, EXPIRATION**

Store the complete kit at 2 – 8 °C. Under these conditions, the kit is stable until the expiration date (see label on the box).

For stability of opened reagents see Chapter 9.
3. INTRODUCTION

Matrix metalloproteinase-8 (MMP-8; called also neutrophil collagenase or collagenase 2) is a member of matrix metalloproteinase family of zinc- and calcium-dependent endo-peptidases responsible for degradation of extracellular matrix. Matrix metalloproteinases (MMPs) possess catalytic properties responsible for tissue remodeling and degradation of structural components of the extracellular matrix (ECM) including collagens, elastins, gelatin, matrix glycoprotein, and proteoglycans [1, 2].

MMP-8 was first described (as neutrophil collagenase) in 1990 when it was cloned from neutrophils obtained from a patient with granulocytic leukemia [3]. Later observations found MMP-8 mRNA expression in chondrocytes as well as in human rheumatoid synovial fibroblasts, activated macrophages, smooth muscle cells and endothelial cells [4, 5]. The human MMP-8 gene is located on chromosome 11q22.3, residing in a gene cluster that contains several MMP genes. Its expression is inducible and upregulated by various inflammatory cytokines, such as interleukin-1β, tumour necrosis factor-α, and CD40 ligand [6]. The MMP-8 protein consists of a signal peptide, a propeptide, a catalytic domain, a hinge region, and a hemopexin-like C-terminal domain [1, 7]. The mature MMP-8 enzyme is 64 kDa in size, with glycosylation increasing the size to 75 kDa. Autoproteolytic degradation has been described, yielding a 40-kDa fragment, which retains catalytic activity but does not cleave fibrillar collagen. MMP-8 can be proteolytically activated also by stromelysin-1 (MMP-3), stromelysin-2 (MMP-10) and by matrilysin-1 (MMP-7) [8-10].

PMN-derived MMP-8 is expressed during the myelocyte stage of development of polymorphonuclear (PMN) precursors in the bone marrow, and it is stored as a latent enzyme (pro-MMP-8) within the specific granules of polymorphonuclear cells. Pro-MMP-8 is rapidly released from activated PMN undergoing degranulation, and is then activated via the cysteine switch mechanism to yield the active form of the enzyme to ensure rapid availability at inflammatory sites [11]. The endogenous MMP inhibitors, TIMPs, can inhibit MMP-8 [12]. Therefore, the activity of MMP-8 in a tissue at a given time would be dependent on the relative amounts of its transcriptional stimuli, zymogen activators and enzymatic inhibitors that are present in that tissue at the time [1].

The best-known substrates of MMP-8 are interstitial collagens (types I-III), the major structural components of the extracellular matrix, among which MMP-8 has higher proteolytic activity on types I and III than type II [1, 13]. In addition, MMP-8 can also cleave nonmatrix proteins such as serpins, bradykinin, angiotensin I, fibrinogen and many other [11]. As a result of its known catalytic activities, MMP-8 is believed to be involved in wound healing and tissue remodeling during inflammation. In addition, MMP-8 has been implicated in the pathogenesis of several chronic inflammatory diseases characterized by excessive influx and activation of polymorphonuclear cell (PMN), including cystic fibrosis, rheumatoid arthritis, chronic skin wounds and periodontal disease [11].

The number of publications investigating the role of MMPs in periodontal disease has been still growing. The imbalance between MMPs and tissue inhibitors of matrix metalloproteinases (TIMPs) is considered to trigger the degradation of extracellular matrix, basement membrane, and alveolar bone, and thus to initiate periodontal disease [14]. MMP-8 was found to be the most prevalent MMP in diseased periodontal tissue, oral fluid, gingival
crevicular fluid (GCF) and saliva. Moreover, MMP-8 activity correlates with disease severity [15]. Reduction of MMP activity was shown to reduce periodontitis progression. MMP-8 is apparently a major mediator of this aggressive tissue destruction, although one report indicates a protective role for MMP-8 during periodontal infection [15, 16].

Several cross-sectional case-control studies have shown that circulating MMP-8 levels are elevated in patients with coronary or carotid atherosclerosis as compared with individuals without evidence of atherosclerosis in the respective artery [1, 17]. MMP-8 protein has been shown to accumulate in atherosclerotic plaques, predominantly in their shoulder region, a site that is most frequently found to rupture [6]. MMP-8 colocalized with cleaved but not intact type I collagen at these sites. Moreover, authors of cited study showed that atherosclerotic plaques with rupture-prone characteristics (a large lipid core and a thin fibrous cap) expressed more MMP-8 than did plaques without these features [1, 6].

MMP-8 was proven to play an essential role in both acute and chronic lung disorders. For instance, in COPD patients, enhanced neutrophil-derived MMP-8 and MMP-9 activity may account for inappropriate ECM remodelling and basement membrane disruption, and for alveolar cell apoptosis [15, 18]. Interestingly, both tumourigenic and antitumourigenic properties have been ascribed to MMP-8 activity. Whether MMP-8 is protective or detrimental seems to depend on the type of tumour and its stage of development and progression [15]. MMP-8 was also reported as capable to prevent metastasis formation through the modulation of tumor cell adhesion and invasion. Analysis of MMP-8 in breast cancer patients revealed that the expression of this metalloproteinase by breast tumors correlates with a lower incidence of lymph node metastasis and confers good prognosis to these patients [19].

MMP-8 is a promising candidate for predicting the progression of some of aforementioned disorders. During periodontal disease, MMP-8 activity is associated with disease severity. As MMP-8 can be easily measured in oral fluids and serum, it has strong potential as a non-invasive, sensitive biomarker of disease progression and response to therapy. Several user-friendly, chair-side diagnostic methods have been developed for the quantification of MMP-8 levels in oral fluids [15].

Urinary MMP-8 activity also correlates with the severity of diabetic nephropathy, and so it might be a useful early marker of diabetic renal disease as well. Additionally, MMP-8 expression in breast cancer is an indicator of reduced risk of axillary lymph node metastasis. MMP-8 was also reported to be a good biomarker for cardiovascular disease because its increased tissue activity and plasma and serum levels are correlated with atherosclerotic plaque vulnerability. However, one should be aware that processing of blood samples during serum preparation significantly influences the accuracy of the measurement of circulating MMPs [15].

Areas of investigation:
Infection and inflammatory disease
Periodontal disease
Cardiovascular disease, atherosclerosis
Preterm labor; intra-amniotic inflammation
Pulmonary disease; COPD, pulmonary fibrosis
Oncology

Page 5 of 28
4. TEST PRINCIPLE

In the BioVendor Human Matrix Metalloproteinase-8 ELISA, standards and samples are incubated in microtitration wells pre-coated with monoclonal anti-human MMP-8 antibody. After 60-minute incubation followed by washing, a different biotin-labelled monoclonal anti-human MMP-8 antibody is added and incubated with the captured MMP-8 for 60 minutes. After another washing, streptavidin-HRP conjugate is added. After 30-minute incubation and the last washing step, the remaining conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of MMP-8. A standard curve is constructed by plotting absorbance values against MMP-8 concentrations of standards and concentrations of unknown samples are determined using this standard curve.
5. PRECAUTIONS

- For professional use only
- Notice: Wear gloves, face mask (or another mouth covering) and laboratory coat when handling ELISA components and during ELISA assay. Saliva may contain MMP-8 and contamination in any ELISA step could cause false positive results
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary
- The materials must not be pipetted by mouth

6. TECHNICAL HINTS

- Reagents with different lot numbers should not be mixed
- Use thoroughly clean glassware
- Use deionized (distilled) water, stored in clean containers
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent
- Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light
- Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements
7. REAGENT SUPPLIED

<table>
<thead>
<tr>
<th>Kit Components</th>
<th>State</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody Coated Microtiter Strips</td>
<td>ready to use</td>
<td>96 wells</td>
</tr>
<tr>
<td>Biotin Labelled Antibody (lyophil.)</td>
<td>lyophilized</td>
<td>2 vials</td>
</tr>
<tr>
<td>Streptavidin-HRP Conjugate</td>
<td>ready to use</td>
<td>13 ml</td>
</tr>
<tr>
<td>Master Standard</td>
<td>lyophilized</td>
<td>2 vials</td>
</tr>
<tr>
<td>Biotin-Ab Diluent</td>
<td>ready to use</td>
<td>13 ml</td>
</tr>
<tr>
<td>Dilution Buffer</td>
<td>ready to use</td>
<td>20 ml</td>
</tr>
<tr>
<td>Wash Solution Conc. (10x)</td>
<td>concentrated</td>
<td>100 ml</td>
</tr>
<tr>
<td>Substrate Solution</td>
<td>ready to use</td>
<td>13 ml</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>ready to use</td>
<td>13 ml</td>
</tr>
<tr>
<td>Product Data Sheet + Certificate of Analysis</td>
<td>-</td>
<td>1 pc</td>
</tr>
</tbody>
</table>

8. MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Precision pipettes to deliver 10 – 1000 µl with disposable tips
- Multichannel pipette to deliver 100 µl with disposable tips
- Plate cover
- Absorbent material (e.g. paper towels) for blotting the microtiter plate after washing
- Vortex mixer
- Orbital microplate shaker capable of approximately 300 rpm
- Microplate washer (optional). [Manual washing is possible but not preferable.]
- Microplate reader with 450 ± 10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550 – 650 nm)
- Software package facilitating data generation and analysis (optional)
9. PREPARATION OF REAGENTS

All reagents need to be brought to room temperature prior to use
Always prepare only the appropriate quantity of reagents for your test
Do not use components after the expiration date marked on their label

- Assay reagents supplied ready to use:

Antibody Coated Microtiter Strips
Stability and storage:
Return the unused strips to the provided aluminium zip-sealed bag with desiccant and seal carefully. Remaining Microtiter Strips are stable 3 months stored at 2 - 8 °C and protected from the moisture.

Streptavidin-HRP Conjugate
Dilution Buffer
Biotin-Ab Diluent
Substrate Solution
Stop Solution
Stability and storage:
Opened reagents are stable 3 months when stored at 2 - 8 °C.

- Assay reagents supplied concentrated or lyophilized:

Human MMP-8 Master Standard
Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of standard!!!
Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). The resulting concentration of the human MMP-8 in the stock solution is 10 ng/ml.

Prepare set of standards using Dilution Buffer as follows:

<table>
<thead>
<tr>
<th>Volume of Standard</th>
<th>Dilution Buffer</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock</td>
<td>-</td>
<td>10 ng/ml</td>
</tr>
<tr>
<td>250 µl of stock</td>
<td>250 µl</td>
<td>5 ng/ml</td>
</tr>
<tr>
<td>250 µl of 5 ng/ml</td>
<td>250 µl</td>
<td>2.5 ng/ml</td>
</tr>
<tr>
<td>250 µl of 2.5 ng/ml</td>
<td>250 µl</td>
<td>1.25 ng/ml</td>
</tr>
<tr>
<td>250 µl of 1.25 ng/ml</td>
<td>250 µl</td>
<td>0.625 ng/ml</td>
</tr>
<tr>
<td>250 µl of 0.625 ng/ml</td>
<td>250 µl</td>
<td>0.312 ng/ml</td>
</tr>
<tr>
<td>250 µl of 0.312 ng/ml</td>
<td>250 µl</td>
<td>0.156 ng/ml</td>
</tr>
</tbody>
</table>
Prepared Standards are ready to use, do not dilute them.

**Stability and storage:**

Do not store the reconstituted and/or diluted Standard solutions.

### Biotin Labelled Antibody

Refer to the Certificate of Analysis for current volume of Biotin-Ab Diluent needed for reconstitution of Biotin Labelled Antibody!!!

Reconstitute the lyophilized Biotin Labelled Antibody with Biotin-Ab Diluent just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).

Dilute Biotin Labelled Antibody Concentrate **100x** with Biotin-Ab Diluent (e.g. 10 µl of Biotin Labelled Antibody Concentrate + 990 µl of Biotin-Ab Diluent for 8 wells).

**Stability and storage:**

Do not store diluted Biotin Labelled Antibody working solution.

### Wash Solution Conc. (10x)

Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution. Example: 100 ml of Wash Solution Concentrate (10x) + 900 ml of distilled water for use of all 96-wells.

**Stability and storage:**

The diluted Wash Solution is stable 1 month when stored at 2 - 8 °C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2 - 8 °C.
10. PREPARATION OF SAMPLES

Serum*, plasma (EDTA, citrate, heparin) and saliva samples were tested with the kit; please see chapter 13 for effect of sample matrix on MMP-8 levels.

Samples can be assayed immediately after collection, or after long-term storage. Thoroughly mix thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

An appropriate dilution should be assessed by the researcher in advance to batch measurement.

Recommended starting dilution for serum, plasma and saliva samples is 5x.
Dilute samples (serum, plasma, saliva) 5x with Dilution Buffer just prior to the assay, e.g. 25 µl of sample + 100 µl of Dilution Buffer for singlets, or preferably 50 µl of sample + 200 µl of Dilution Buffer for duplicates. Mix well (not to foam). Vortex is recommended.

Stability and storage:
Samples should be stored at -20 °C, or preferably at -70 °C or lower for long-term storage. Avoid repeated freeze/thaw cycles.
Do not store the diluted samples.

Note: It is recommended to use a precision pipette and a careful technique to perform the dilution to get precise results.

Ask for details at info@biovendor.com if assaying amniotic fluid, bronchoalveolar lavage fluid, or urine.

*Pay attention to processing of blood specimens, especially if using serum collection tubes, as obtained MMP-8 levels may be dependent on the duration of coagulation process. We recommend not to use serum collection tubes with clotting activator since it has been shown that the samples with clotting activator provide MMP-8 concentration values markedly higher than serum samples collected without the clotting activator. Strictly follow the manufacturer’s instructions for correct handling of given tubes [20-22].
11. ASSAY PROCEDURE

1. Pipet 100 \( \mu l \) of diluted standards, Dilution Buffer (= Blank) and diluted samples, preferably in duplicates, into the appropriate wells. See Figure 1 for example of worksheet.
2. Incubate the plate at room temperature (ca. 25 °C) for 1 hour, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 5-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Add 100 \( \mu l \) of Biotin Labelled Antibody solution into each well.
5. Incubate the plate at room temperature (ca. 25 °C) for 1 hour, shaking at ca. 300 rpm on an orbital microplate shaker.
6. Wash the wells 5-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add 100 \( \mu l \) of Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at room temperature (ca. 25 °C) for 30 min, shaking at ca. 300 rpm on an orbital microplate shaker.
9. Wash the wells 5-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
10. Add 100 \( \mu l \) of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
11. Incubate the plate for 10 minutes at room temperature. The incubation time may be extended [up to 25 minutes] if the reaction temperature is below than 20 °C. Do not shake the plate during the incubation.
12. Stop the colour development by adding 100 \( \mu l \) of Stop Solution.
13. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 - 650 nm). Subtract readings at 630 nm (550 - 650 nm) from the readings at 450 nm.

The absorbance should be read within 5 minutes following step 12.

Note: If some samples and standard/s have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine MMP-8 concentration of off-scale standards and samples. The readings at 405 nm should not replace the readings for samples that were “in range” at 450 nm.

Note 2: Manual washing 5-times: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat four times. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.
<table>
<thead>
<tr>
<th></th>
<th>strip 1+2</th>
<th>strip 3+4</th>
<th>strip 5+6</th>
<th>strip 7+8</th>
<th>strip 9+10</th>
<th>strip 11+12</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Standard 10</td>
<td>Sample 1</td>
<td>Sample 9</td>
<td>Sample 17</td>
<td>Sample 25</td>
<td>Sample 33</td>
</tr>
<tr>
<td>B</td>
<td>Standard 5</td>
<td>Sample 2</td>
<td>Sample 10</td>
<td>Sample 18</td>
<td>Sample 26</td>
<td>Sample 34</td>
</tr>
<tr>
<td>C</td>
<td>Standard 2.5</td>
<td>Sample 3</td>
<td>Sample 11</td>
<td>Sample 19</td>
<td>Sample 27</td>
<td>Sample 35</td>
</tr>
<tr>
<td>D</td>
<td>Standard 1.25</td>
<td>Sample 4</td>
<td>Sample 12</td>
<td>Sample 20</td>
<td>Sample 28</td>
<td>Sample 36</td>
</tr>
<tr>
<td>E</td>
<td>Standard 0.62</td>
<td>Sample 5</td>
<td>Sample 13</td>
<td>Sample 21</td>
<td>Sample 29</td>
<td>Sample 37</td>
</tr>
<tr>
<td>F</td>
<td>Standard 0.31</td>
<td>Sample 6</td>
<td>Sample 14</td>
<td>Sample 22</td>
<td>Sample 30</td>
<td>Sample 38</td>
</tr>
<tr>
<td>G</td>
<td>Standard 0.15</td>
<td>Sample 7</td>
<td>Sample 15</td>
<td>Sample 23</td>
<td>Sample 31</td>
<td>Sample 39</td>
</tr>
<tr>
<td>H</td>
<td>Blank</td>
<td>Sample 8</td>
<td>Sample 16</td>
<td>Sample 24</td>
<td>Sample 32</td>
<td>Sample 40</td>
</tr>
</tbody>
</table>

*Figure 1: Example of a work sheet.*
Most microtiter plate readers perform automatic calculations of analyte concentration. The Standards curve is constructed by plotting the mean absorbance of Standards (Y) against the known concentration of Standards (X) in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of MMP-8 (ng/ml) in samples.

Alternatively, the *logit log* function can be used to linearize the standard curve, i.e. *logit* of the mean absorbance (Y) is plotted against *log* of the known concentration of Standards (X).

The measured concentration of samples calculated from the standard curve must be multiplied by their respective dilution factor, because samples have been diluted prior to the assay, e.g. 0.63 ng/ml (from standard curve) x 5 (dilution factor) = 3.15 ng/ml.

![Human MMP-8 ELISA Standard Curve](image)

*Figure 2: Typical standard curve for Human MMP-8 ELISA.*
13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human MMP-8 ELISA are presented in this chapter

- **Sensitivity**
  Limit of detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: $A_{\text{blank}} + 3 \times \text{SD}_{\text{blank}}$) is calculated from the real human MMP-8 values in wells and is: 0.025 ng/ml.
  * Dilution Buffer is pipetted into blank wells.

- **Limit of Assay**
  Samples with absorbances exceeding the absorbance of the highest standard should be measured again with higher dilution. The final concentration of samples calculated from the standard curve must be multiplied by the respective dilution factor.

- **Specificity**
  The antibodies used in this ELISA are specific to human MMP-8 with no significant cross reactivities to MMP-2, MMP-3, MMP-9, and TIMP-1 (all at 100 ng/ml) in Human MMP-8 ELISA.

Presented results are multiplied by respective dilution factor

- **Precision**
  Intra-assay (Within-Run) (n=8)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (ng/ml)</th>
<th>SD (ng/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.18</td>
<td>0.14</td>
<td>2.7</td>
</tr>
<tr>
<td>2</td>
<td>22.01</td>
<td>0.79</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Inter-assay (Run-to-Run) (n=7)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (ng/ml)</th>
<th>SD (ng/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.62</td>
<td>0.39</td>
<td>6.9</td>
</tr>
<tr>
<td>2</td>
<td>31.97</td>
<td>2.60</td>
<td>8.1</td>
</tr>
</tbody>
</table>
• **Spiking Recovery**
Serum samples were diluted, spiked with different amounts of human MMP-8 and assayed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Observed (ng/ml)</th>
<th>Expected (ng/ml)</th>
<th>Recovery O/E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>5.37</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>9.24</td>
<td>8.50</td>
<td>108.7</td>
</tr>
<tr>
<td></td>
<td>12.81</td>
<td>11.62</td>
<td>110.2</td>
</tr>
<tr>
<td></td>
<td>20.15</td>
<td>17.87</td>
<td>112.7</td>
</tr>
<tr>
<td>Serum 2</td>
<td>13.98</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>19.13</td>
<td>17.10</td>
<td>111.8</td>
</tr>
<tr>
<td></td>
<td>20.64</td>
<td>20.23</td>
<td>102.0</td>
</tr>
<tr>
<td></td>
<td>26.69</td>
<td>26.48</td>
<td>100.8</td>
</tr>
</tbody>
</table>

• **Linearity**
Serum and saliva samples were serially diluted with Dilution Buffer and assayed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Observed (ng/ml)</th>
<th>Expected (ng/ml)</th>
<th>Recovery O/E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>-</td>
<td>17.99</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>9.87</td>
<td>9.00</td>
<td>109.7</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>4.94</td>
<td>4.50</td>
<td>109.8</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>2.28</td>
<td>2.25</td>
<td>101.4</td>
</tr>
<tr>
<td>Serum 2</td>
<td>-</td>
<td>28.01</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>14.12</td>
<td>14.00</td>
<td>100.8</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>7.04</td>
<td>7.00</td>
<td>100.5</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>3.45</td>
<td>3.50</td>
<td>98.5</td>
</tr>
<tr>
<td>Saliva 1</td>
<td>-</td>
<td>39.36</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>18.14</td>
<td>19.68</td>
<td>92.2</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>11.47</td>
<td>9.84</td>
<td>116.5</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>5.39</td>
<td>4.92</td>
<td>109.5</td>
</tr>
<tr>
<td>Saliva 2</td>
<td>-</td>
<td>104.20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>57.48</td>
<td>52.1</td>
<td>110.3</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>25.52</td>
<td>26.1</td>
<td>98.0</td>
</tr>
</tbody>
</table>
Effect of sample matrix

EDTA, citrate and heparin plasma samples were compared to respective serum samples from the same 10 individuals. Results are shown below:

<table>
<thead>
<tr>
<th>Volunteer No.</th>
<th>Serum (ng/ml)</th>
<th>Plasma (ng/ml)</th>
<th>EDTA</th>
<th>Citrate</th>
<th>Heparin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26.24</td>
<td>6.68</td>
<td>3.20</td>
<td>14.78</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>26.03</td>
<td>6.23</td>
<td>2.67</td>
<td>10.53</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>45.84</td>
<td>6.73</td>
<td>1.40</td>
<td>4.42</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>52.50</td>
<td>6.79</td>
<td>7.15</td>
<td>5.10</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>32.50</td>
<td>11.79</td>
<td>8.04</td>
<td>4.22</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>28.62</td>
<td>4.00</td>
<td>1.62</td>
<td>3.34</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>41.18</td>
<td>3.80</td>
<td>3.07</td>
<td>12.80</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>17.50</td>
<td>3.39</td>
<td>1.20</td>
<td>1.65</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>52.50</td>
<td>7.09</td>
<td>2.87</td>
<td>16.46</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>23.72</td>
<td>6.13</td>
<td>3.35</td>
<td>4.45</td>
<td></td>
</tr>
</tbody>
</table>

Mean (ng/ml) 34.66  6.26  3.46  7.77

Mean Plasma/Serum (%)

Coefficient of determination $R^2$

<table>
<thead>
<tr>
<th></th>
<th>Serum</th>
<th>EDTA Plasma</th>
<th>Citrate Plasma</th>
<th>Heparin Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volunteers</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Concentration of MMP-8 (ng/ml)</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
</tbody>
</table>

Figure 3: MMP-8 levels measured using Human MMP-8 ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.
14. DEFINITION OF THE STANDARD

The recombinant human MMP-8 is used as the standard. The recombinant MMP-8 produced in human cell culture is a 52 kDa protein consisting of 448 amino acid residues of human MMP-8.

15. PRELIMINARY POPULATION DATA

The following results were obtained when serum samples from 155 unselected donors (89 men + 66 women) 21 - 65 years old were assayed with the BioVendor Human MMP-8 ELISA in our laboratory.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (years)</th>
<th>n</th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>20-29</td>
<td>18</td>
<td>17.05</td>
<td>13.44</td>
<td>10.88</td>
<td>4.86</td>
<td>48.88</td>
</tr>
<tr>
<td></td>
<td>30-39</td>
<td>26</td>
<td>18.40</td>
<td>15.43</td>
<td>11.55</td>
<td>6.08</td>
<td>59.22</td>
</tr>
<tr>
<td></td>
<td>40-49</td>
<td>31</td>
<td>20.44</td>
<td>19.03</td>
<td>9.98</td>
<td>5.01</td>
<td>55.50</td>
</tr>
<tr>
<td></td>
<td>50-65</td>
<td>14</td>
<td>22.64</td>
<td>22.08</td>
<td>7.37</td>
<td>6.78</td>
<td>34.63</td>
</tr>
<tr>
<td>Women</td>
<td>20-29</td>
<td>12</td>
<td>22.20</td>
<td>20.95</td>
<td>8.68</td>
<td>10.32</td>
<td>40.26</td>
</tr>
<tr>
<td></td>
<td>30-39</td>
<td>26</td>
<td>21.67</td>
<td>17.97</td>
<td>12.28</td>
<td>6.97</td>
<td>59.31</td>
</tr>
<tr>
<td></td>
<td>40-49</td>
<td>20</td>
<td>19.98</td>
<td>18.05</td>
<td>8.25</td>
<td>10.16</td>
<td>42.28</td>
</tr>
<tr>
<td></td>
<td>50-61</td>
<td>8</td>
<td>14.27</td>
<td>12.35</td>
<td>7.38</td>
<td>6.12</td>
<td>30.93</td>
</tr>
</tbody>
</table>

- **Reference range**
  It is recommended that each laboratory include its own panel of control samples in the assay. Each laboratory should establish its own normal and pathological reference ranges for MMP-8 levels with the assay.
16. TROUBLESHOOTING AND FAQS

Weak signal in all wells
Possible explanations:
- Omission of a reagent or a step
- Improper preparation or storage of a reagent
- Assay performed before reagents were allowed to come to room temperature
- Improper wavelength when reading absorbance

High signal and background in all wells
Possible explanations:
- Improper or inadequate washing
- Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
- Incubation temperature over 30 °C

High coefficient of variation (CV)
Possible explanation:
- Improper or inadequate washing
- Improper mixing Standards or samples
17. REFERENCES

References to MMP-8:


For more references on this product see our WebPages at www.biovendor.com
## 18. EXPLANATION OF SYMBOLS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>REF</td>
<td>Catalogue number</td>
</tr>
<tr>
<td>Cont.</td>
<td>Content</td>
</tr>
<tr>
<td>LOT</td>
<td>Lot number</td>
</tr>
<tr>
<td>!</td>
<td>Attention, see instructions for use</td>
</tr>
<tr>
<td>bio</td>
<td>Potential biological hazard</td>
</tr>
<tr>
<td></td>
<td>Expiry date</td>
</tr>
<tr>
<td>8 °C</td>
<td>Storage conditions</td>
</tr>
<tr>
<td></td>
<td>Name and registered office of the manufacturer</td>
</tr>
</tbody>
</table>
**Assay Procedure Summary**

1. **Reconstitute Master Standard and prepare set of standards**

2. **Add standards, and samples 100 µl**

3. **Wash 5x**

4. **Add Biotin Labelled Antibody solution 100 µl**

5. **Wash 5x**

6. **Add Streptavidin-HRP Conjugate 100 µl**

7. **Wash 5x**

8. **Add Substrate Solution 100 µl**

9. **Add Stop Solution 100 µl**

10. **Read absorbance and calculate results**

11. **Dilute samples (5x)**

   - Incubate at RT for 1 hour / 300 rpm
   - Incubate at RT for 1 hour / 300 rpm
   - Incubate at RT for 30 min / 300 rpm
   - Incubate at RT for 10 min