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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 RD191439100CS Human Matrix Metalloproteinase-9 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human matrix metalloproteinase-9 (MMP 9).

Features

- It is intended for research use only
- The total assay time is less than 3 hours
- The kit measures human matrix metalloproteinase-9 in human serum, plasma (EDTA, heparin), saliva and urine.
- 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 metalloproteinases (MMPs) are a group of enzymes engaged in the degradation and remodeling of extracellular matrix (ECM). Nowadays six groups of these enzymes have been distinguished (collagenases, gelatinases, stromelysins, matrilysins, membrane-type, and a sixth group encompassing several other MMPs not classified in the previous categories), differing in structure, cellular localization, and substrate specificity. Since these enzymes are involved in connective tissue remodeling occurring in the course of morphogenetic processes, therefore, they are a subject of a very strict regulation, which is executed, among others, by the expression of their specific inhibitors—tissue inhibitors of metalloproteinases (TIMPs).\(^1\)

MMPs 2 and 9 are named type IV collagenases, or alternatively gelatinase A and B, respectively. Their degrading substrates are gelatine, the denatured form of collagen, and type IV collagen, the main component of the basement membrane.\(^2\)

One of the members of the MMP family, MMP-9, is a gelatinase that has been implicated in the pathogenesis of atherosclerosis and chronic obstructive pulmonary disease (COPD) in addition to tumor formation and metastasis. Accordingly, a number of studies have associated elevated serum levels of MMP-9 with many chronic inflammatory conditions including coronary artery disease (CAD), COPD, arthritis and metabolic syndrome.\(^3\)

Notably, high levels of MMP-9 have been associated with plaque progression, destabilization and rupture. These various effects exaggerate the inflammatory process, promoting atherosclerosis and increasing the risk of atherothrombosis and cardiovascular (CV) events.\(^4\)

Thus, MMP-9 has emerged as a novel disease marker as well as a therapeutic target.\(^3\)

MMP9, like other MMPs, belongs to a superfamily of zinc containing proteases and has been shown to associate with tumorigenesis.\(^5\)

Overexpression of tissue MMPs has been correlated with progression in many tumour types, and overexpression of MMP9 has been found in colorectal adenomas and carcinomas. A significant positive correlation has also been found between tissue MMP9 and the stage of colorectal tumours at diagnosis.\(^6\)

Elevated expression of MMP-9, along with MMP-2 is usually seen in invasive and highly tumorigenic cancers such as colorectal tumors, gastric carcinoma, pancreatic carcinoma, breast cancer, oral cancer, melanoma, malignant gliomas, chondrosarcoma, gastrointestinal adenocarcinoma. Levels are also increased in malignant astrocytomas, carcinomatous meningitis, and brain metastases.\(^7\)

Areas of investigation:
Multiple sclerosis
Inflammatory diseases
Cancer
4. TEST PRINCIPLE

In the BioVendor Human Matrix Metalloproteinase 9 ELISA, standards and samples are incubated in microplate wells pre-coated with polyclonal anti-human MMP 9 antibody. After 60 minutes incubation and washing, polyclonal anti-human MMP-9 antibody, conjugated with horseradish peroxidase (HRP) is added to the wells and incubated for 60 minutes with captured MMP-9. Following another washing step, the remaining HRP 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 spectrophotometrically at 450 nm. The absorbance is proportional to the concentration of MMP-9. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

5. PRECAUTIONS

- For professional use only
- Wear gloves and laboratory coats when handling immunodiagnostic materials
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled
- This kit may contain components of human or animal origin. These materials should be handled as potentially infectious
- 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. REAGENTS 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>Conjugate Solution</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>Dilution Buffer</td>
<td>ready to use</td>
<td>2x13 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.

  Conjugate Solution
  Dilution Buffer
  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-9 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-9 in the stock solution is **20 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>20 ng/ml</td>
</tr>
<tr>
<td>250 µl of stock</td>
<td>250 µl</td>
<td>10 ng/ml</td>
</tr>
<tr>
<td>250 µl of 10 ng/ml</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>0.63 ng/ml</td>
</tr>
<tr>
<td>250 µl of 0.63 ng/ml</td>
<td>250 µl</td>
<td>0.31 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.

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

The kit measures human MMP-9 in serum*, plasma**, saliva and urine.

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 is 50x.**
Dilute samples (serum, plasma) 50x with Dilution Buffer just prior to the assay, e.g. 5 µl of sample + 245 µl of Dilution Buffer for singlets or duplicates. **Mix well** (not to foam). Vortex is recommended.

**Recommended starting dilution for urine is 4x.**
Dilute samples (serum, plasma) 4x with Dilution Buffer just prior to the assay, e.g. 30 µl of sample + 90 µl of Dilution Buffer for singlets or e.g. 60 µl of sample + 180 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.**

See Chapter 13 for effect of sample matrix (serum/plasma) on the concentration of human matrix metalloproteinase 9.

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

*Pay attention to a possibly elevated serum level of human MMP-9 due to MMP-9 release by platelets during platelet activation (sampling process).

** EDTA and citrate using are not recommended anticoagulants for use in this assay due to their chelating properties.
11. ASSAY PROCEDURE

1. Pipet 100 μl of diluted standards, Dilution Buffer (= Blank) and diluted samples, preferably in duplicates, into the appropriate wells. See Figure 1 for example of work sheet.
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 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Add 100 μl Conjugate 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 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add 100 μl of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
8. Incubate the plate for 10 minutes at room temperature. The incubation time may be extended if the reaction temperature is below than 20 °C. Do not shake the plate during the incubation.
9. Stop the colour development by adding 100 μl of Stop Solution.
10. 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 10.

Note: If some samples and standard/s have absorbance 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 matrix metalloproteinase-9 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 20.0</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 10.0</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 5.0</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 2.5</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 1.250</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 0.625</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 0.313</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.
12. CALCULATIONS

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-9 (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. 25 ng/ml (from standard curve) x 50 (dilution factor) = 1 250 ng/ml.

![Human MMP9 ELISA Standard Curve](image)

*Figure 2: Typical standard curve for Human MMP-9 ELISA.*
13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human MMP-9 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-9 values in wells and is: 0.151 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 matrix metalloproteinase-9 with no significant crossreactivities to recombinant matrix metalloproteinase-3, matrix metalloproteinase-8 or TIMP-1 (all at 20 ng/ml) in Human Matrix Metalloproteinase-9ELISA.

**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>285.80</td>
<td>5.90</td>
<td>2.1</td>
</tr>
<tr>
<td>2</td>
<td>174.51</td>
<td>3.54</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Inter-assay (Run-to-Run) (n=6)

<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>171.78</td>
<td>7.14</td>
<td>4.2</td>
</tr>
<tr>
<td>2</td>
<td>145.49</td>
<td>7.26</td>
<td>5.0</td>
</tr>
</tbody>
</table>
Spiking Recovery

Serum samples were diluted, spiked with different amounts of human MMP-9 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>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>233</td>
<td>225</td>
<td>103.6</td>
</tr>
<tr>
<td></td>
<td>360</td>
<td>350</td>
<td>102.8</td>
</tr>
<tr>
<td></td>
<td>630</td>
<td>600</td>
<td>105.0</td>
</tr>
<tr>
<td>Serum 2</td>
<td>98</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>236</td>
<td>223</td>
<td>105.7</td>
</tr>
<tr>
<td></td>
<td>363</td>
<td>348</td>
<td>104.4</td>
</tr>
<tr>
<td></td>
<td>624</td>
<td>598</td>
<td>104.4</td>
</tr>
</tbody>
</table>

Linearity

Serum 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>273</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>135</td>
<td>136</td>
<td>98.8</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>66</td>
<td>68</td>
<td>97.4</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>31</td>
<td>34</td>
<td>92.1</td>
</tr>
<tr>
<td>Serum 2</td>
<td>-</td>
<td>238</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>117</td>
<td>113</td>
<td>102.1</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>58</td>
<td>56</td>
<td>103.0</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>27</td>
<td>28</td>
<td>96.9</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>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EDTA</td>
</tr>
<tr>
<td>1</td>
<td>271</td>
<td>106</td>
</tr>
<tr>
<td>2</td>
<td>510</td>
<td>86</td>
</tr>
<tr>
<td>3</td>
<td>293</td>
<td>84</td>
</tr>
<tr>
<td>4</td>
<td>605</td>
<td>94</td>
</tr>
<tr>
<td>5</td>
<td>529</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>382</td>
<td>30</td>
</tr>
<tr>
<td>7</td>
<td>509</td>
<td>84</td>
</tr>
<tr>
<td>8</td>
<td>589</td>
<td>128</td>
</tr>
<tr>
<td>9</td>
<td>436</td>
<td>79</td>
</tr>
<tr>
<td>10</td>
<td>69</td>
<td>36</td>
</tr>
</tbody>
</table>

Mean (ng/ml) | 419.32 | 82.8 | 26.9 | 64.8 |

Mean Plasma/Serum (%) | 19.7 | 6.4 | 15.5 |

Coefficient of determination R² | 0.35 | 0.25 | 0.05 |

Figure 3: Matrix metalloproteinase-9 (MMP-9) levels measured using Human Matrix metalloproteinase-9 (MMP-9) ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.
14. DEFINITION OF THE STANDARD

The recombinant human matrix metalloproteinase-9 (MMP-9) is used as the standard. The recombinant matrix metalloproteinase-9 (MMP-9) expressed in HEK293 is a 77 kDa protein consisting of 688 amino acid residues of human matrix metalloproteinase-9 (MMP-9).

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 Matrix metalloproteinase-9 (MMP-9) ELISA in our laboratory.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (years)</th>
<th>n</th>
<th>MMP-9 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Men</td>
<td>20-29</td>
<td>18</td>
<td>240</td>
</tr>
<tr>
<td></td>
<td>30-39</td>
<td>26</td>
<td>281</td>
</tr>
<tr>
<td></td>
<td>40-49</td>
<td>31</td>
<td>262</td>
</tr>
<tr>
<td></td>
<td>50-65</td>
<td>14</td>
<td>316</td>
</tr>
<tr>
<td>Women</td>
<td>20-29</td>
<td>12</td>
<td>261</td>
</tr>
<tr>
<td></td>
<td>30-39</td>
<td>26</td>
<td>255</td>
</tr>
<tr>
<td></td>
<td>40-49</td>
<td>20</td>
<td>263</td>
</tr>
<tr>
<td></td>
<td>50-61</td>
<td>8</td>
<td>158</td>
</tr>
</tbody>
</table>

- **Reference range**
  It is recommended that each laboratory include its own panel of control sample in the assay. Each laboratory should establish its own normal and pathological reference ranges for matrix metalloproteinase-9 (MMP-9) levels with the assay.
Figure 4: Human matrix metalloproteinase-9 (MMP-9) concentration plotted against donor age and sex.

16. TROUBLESHOOTING AND FAQS

Strong 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 matrix metalloproteinase 9 (MMP-9):


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>See instructions for use</td>
</tr>
<tr>
<td><img src="image" alt="Biological hazard" /></td>
<td>Biological hazard</td>
</tr>
<tr>
<td><img src="image" alt="Expiry date" /></td>
<td>Expiry date</td>
</tr>
<tr>
<td>2 °C &lt; 8 °C</td>
<td>Storage conditions</td>
</tr>
<tr>
<td><img src="image" alt="Identification of packaging materials" /></td>
<td>Identification of packaging materials</td>
</tr>
</tbody>
</table>
Antibody Coated Microtiter Plate

Reconstitute Master Standard and prepare set of standards

Add standards, and samples 100 μl

Prepare Wash Solution

Add Conjugate Solution 100 μl

Wash 3x

Add Substrate Solution 100 μl

Add Stop Solution 100 μl

Incubate at RT for 1 hour / 300 rpm

Wash 3x

Incubate at RT for 10 min

Read absorbance and calculate results

Dilute samples (50x)
BioVendor – Laboratorni medicina a.s.
Karasek 1767/1, 621 00 Brno, Czech Republic
Phone: +420-549-124-185, Fax: +420-549-211-460
E-mail: info@biovendor.com, sales@biovendor.com
Web: www.biovendor.com

There are BioVendor branches and distributors near you.
To find the office closest to you, visit www.biovendor.com/contact