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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 RD191328200R Human Apoptosis Inhibitor of Macrophage ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human AIM.

Features

- For research use only!
- The total assay time is less than 4 hours
- The kit measures total AIM in serum and plasma (heparin)
- Assay format is 96 wells
- Standard is recombinant protein
- 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.
CD5 molecule-like protein (CD5L), also known as apoptosis inhibitor expressed by macrophages (AIM), is a 50 kDa alternative cell surface ligand for CD5, a glycoprotein expressed on T lymphocytes (1). It is a soluble glycosylated protein that belongs to group B scavenger receptor cysteine-rich (SRCR) superfamily (2). Its expression was detected in the macrophages present in several lymphoid tissues (3). It is widely known, that oxLDL is an inducer of AIM expression. Biochemical studies of this molecule revealed that it is an abundant serum protein and might play a role in the homeostasis of IgM antibodies (2) because CD5L was found to circulate in serum mostly in complex with IgM (4). A strong correlation between AIM and natural IgM levels in the blood has been found in both humans and mice. CD5L has been demonstrated to support the survival of macrophages and enhanced the phagocytic function of macrophages. In mice, it was also shown to inhibit the apoptosis of NKT cells and T cells (4). CD5L is up-regulated in macrophages at inflammatory sites (4, 6). Increased level of CD5L protects foam cells from apoptosis but permits more rapid cellular accumulation and atherosclerotic plaque formation (7).

AIM is incorporated into adipocytes via endocytosis mediated by the CD 36 scavenger receptor (8) and induces lipolysis via the reduction of FAS enzymatic activity. This decreases lipid droplet storage within adipocytes (9) and their size. On the other hand, when this lipolytic effect is excessive, it triggers chronic inflammation via the recruitment of macrophages into adipose tissue, leading to insulin resistance (IR) (9). During early periods of MetS AIM can help prevent the progression of obesity through lipolysis; in obese conditions, anti-AIM therapy should prevent the development of metabolic diseases such as diabetes and cardiovascular events. One of the criteria for assessing whether AIM or anti-AIM therapy should be administered is the blood AIM level (9).

As written above, most of circulating AIM is associated with IgM pentamers. A large proportion of natural IgM is polyreactive to not only foreign antigens but also autoantigens, including nucleic acids, heat shock proteins, carbohydrates, and phospholipids (10-12). Thus, IgM is believed to be important for the progression of autoimmunity.

One of the main diseases underlying chronic kidney disease (CKD) is nephrosclerosis (13), which involves progressive arteriosclerosis at the level of small arteries and arterioles in the kidneys leading to ischemic changes in the glomeruli and interstitium, consequently compromising renal function (14). As was previously reported, macrophages play a major role in the progression of arteriosclerosis. Uramatsu and co. showed that AIM expression in macrophages in the renal tissue of stroke-prone spontaneously hypertensive (SHRsp) rats seemed closely correlated with the number of infiltrating macrophages (15). It is well known that glomerular or interstitial macrophage infiltration is a prominent feature in nephrosclerosis, diabetic nephropathy, and lupus nephritis (16).

Areas of investigation:
Metabolic syndrome
Cardiovascular disease
Immune Response, Infection and Inflammation
Renal disease
4. TEST PRINCIPLE

In the BioVendor Human Apoptosis Inhibitor of Macrophage ELISA, standards and samples are incubated in a microtitrate plate wells pre-coated with polyclonal anti-human AIM antibody. After 60 min incubation and a washing, biotin-label led polyclonal anti-human AIM antibody is added and incubated with captured AIM for 60 min. After another washing, the streptavidin-HRP conjugate is added. After 30 min 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 AIM. A standard curve is constructed by plotting absorbance values against concentrations of AIM 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. 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</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>2 x 20 ml</td>
</tr>
<tr>
<td>Wash Solution Conc. (10x)</td>
<td>concentrate</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 5 -1000 μl with disposable tips
- Multichannel pipette to deliver 100 μl with disposable tips
- 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 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
  Biotin-Ab Diluent
  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 AIM 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 (do not foam).
The resulting concentration of AIM 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>10.0 ng/ml</td>
</tr>
<tr>
<td>300 µl of std. 10.0 ng/ml</td>
<td>300 µl</td>
<td>5.0 ng/ml</td>
</tr>
<tr>
<td>300 µl of std. 5.0 ng/ml</td>
<td>300 µl</td>
<td>2.5 ng/ml</td>
</tr>
<tr>
<td>300 µl of std. 2.5 ng/ml</td>
<td>300 µl</td>
<td>1.25 ng/ml</td>
</tr>
<tr>
<td>300 µl of std. 1.25 ng/ml</td>
<td>300 µl</td>
<td>0.63 ng/ml</td>
</tr>
<tr>
<td>300 µl of std. 0.63 ng/ml</td>
<td>300 µl</td>
<td>0.31 ng/ml</td>
</tr>
<tr>
<td>300 µl of std. 0.31 ng/ml</td>
<td>300 µl</td>
<td>0.16 ng/ml</td>
</tr>
</tbody>
</table>

Prepared Standards are ready to use, do not dilute them.

**Stability and storage:**
Reconstituted Master Standard must be used immediately.
**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 reconstituted 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 1 strip (8 wells).

**Stability and storage:**
Reconstituted Biotin Labelled Antibody must be used immediately.
**Do not store the reconstituted and/or diluted Biotin Labelled Antibody solutions.**

**Wash Solution Conc. (10x)**
Dilute Wash Solution Concentrate (10x) ten-fold in 900 ml of distilled water to prepare a 1x working solution, e.g. 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.
The kit measures AIM in serum and plasma (heparin).

Samples should be assayed immediately after collection or should be stored at -20°C. Mix thoroughly thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

**Preparation of samples:**
Dilute samples 500x with Dilution Buffer just prior to the assay in two steps as follows:

**Dilution A (20x):**
10 μl of sample + 190 μl of Dilution Buffer and mix well (not to foam). Vortex is recommended.

**Dilution B (25x):**
10 μl Dilution A + 240 μl of Dilution Buffer and mix well (not to foam). Vortex is recommended.

**Stability and storage:**
Samples should be stored at -20°C, or preferably at -70°C 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 AIM. For use of other sample matrices contact info@biovendor.com.

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

1. Pipet 100 µl of diluted Standards, Dilution Buffer (=Blank) and 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 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against a paper towel.
4. Pipet 100 µl of Biotin Labelled Antibody 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 a paper towel.
7. Pipet 100 µl of Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at room temperature (ca. 25°C) for 30 minutes, shaking at ca. 300 rpm on an orbital microplate shaker.
9. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against a paper towel.
10. 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.
11. Incubate the plate for 10 minutes at room temperature. The incubation time may be extended [up to 20 minutes] if the reaction temperature is less than 20°C. Do not shake the plate during the incubation.
12. Stop the colour development by adding 100 µl 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 AIM 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: 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 10.0</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 5.0</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 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 0.63</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 0.16</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 microplate readers perform automatic calculations of analyte concentration. The Standard curve is constructed by plotting the mean absorbance (Y) of Standards against the known concentration (X) of Standards in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of AIM (ng/ml) in samples.

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

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. 0.45 ng/ml (from standard curve) x 500 (dilution factor) = 225 ng/ml.

![Human AIM ELISA Standard Curve](image)

*Figure 2: Typical Standard Curve for Human AIM ELISA.*
13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human AIM 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 AIM values in wells and is 0.009 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 for human AIM.

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>1866</td>
<td>65.5</td>
<td>3.8</td>
</tr>
<tr>
<td>2</td>
<td>314</td>
<td>9.8</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Inter-assay (Run-to-Run) (n=5)

<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>2622</td>
<td>167.8</td>
<td>6.4</td>
</tr>
<tr>
<td>2</td>
<td>335</td>
<td>19.0</td>
<td>5.7</td>
</tr>
</tbody>
</table>
- **Spiking Recovery**
  Serum samples were spiked with different amounts of human AIM 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>1</td>
<td>207</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>325</td>
<td>309</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>430</td>
<td>450</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>694</td>
<td>682</td>
<td>102</td>
</tr>
<tr>
<td>2</td>
<td>252</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>361</td>
<td>354</td>
<td>102</td>
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<td></td>
<td>474</td>
<td>496</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>711</td>
<td>728</td>
<td>98</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>1</td>
<td>-</td>
<td>1981</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>1026</td>
<td>991</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>501</td>
<td>495</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>263</td>
<td>248</td>
<td>106</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>2992</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>1517</td>
<td>1496</td>
<td>101</td>
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<td></td>
<td>4x</td>
<td>763</td>
<td>748</td>
<td>102</td>
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<tr>
<td></td>
<td>8x</td>
<td>395</td>
<td>374</td>
<td>106</td>
</tr>
</tbody>
</table>
Effect of sample matrix
EDTA, citrate and heparin plasmas 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>1092</td>
<td>1929</td>
<td>2865</td>
<td>1172</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>811</td>
<td>1512</td>
<td>1461</td>
<td>746</td>
<td></td>
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<tr>
<td>3</td>
<td>1475</td>
<td>2576</td>
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<td>1741</td>
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<tr>
<td>4</td>
<td>2388</td>
<td>3909</td>
<td>2826</td>
<td>2251</td>
<td></td>
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<tr>
<td>5</td>
<td>1285</td>
<td>4035</td>
<td>2397</td>
<td>1388</td>
<td></td>
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<tr>
<td>6</td>
<td>1220</td>
<td>4565</td>
<td>3120</td>
<td>1518</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>318</td>
<td>2163</td>
<td>1767</td>
<td>418</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>256</td>
<td>2365</td>
<td>1683</td>
<td>446</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>632</td>
<td>2779</td>
<td>2414</td>
<td>815</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1914</td>
<td>4332</td>
<td>3160</td>
<td>2048</td>
<td></td>
</tr>
<tr>
<td>Mean (ng/ml)</td>
<td>1139</td>
<td>3016</td>
<td>2435</td>
<td>1254</td>
<td></td>
</tr>
<tr>
<td>Mean Plasma/Serum (%)</td>
<td>-</td>
<td>265%</td>
<td>214%</td>
<td>110%</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3: AIM levels measured using Human Apoptosis Inhibitor of Macrophage ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.
14. DEFINITION OF THE STANDARD

The Standard used in this kit is recombinant protein. Recombinant human AIM, produced in HEK cells, is 36.9 kDa protein consisting of 334 amino-acid residues of human AIM and 6 additional amino-acids.

15. PRELIMINARY POPULATION DATA

The following results were obtained when serum samples from 155 unselected donors (89 men + 66 women) 20 - 69 years old were assayed with the Biovendor Human Apoptosis Inhibitor of Macrophage ELISA in our laboratory:

- **Age and Sex dependent distribution of human AIM**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (years)</th>
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Reference range
The data quoted in these instructions should be used for guidance only. 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 human AIM protein levels with the assay.

16. METHOD COMPARISON

The BioVendor Human Apoptosis Inhibitor of Macrophage ELISA has not been compared to any commercial immunoassay.

17. 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
- Manual washing
- 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

High signal of standards and samples
Possible explanations:
- Incubation temperature over 30°C. Performing the incubation at the temperature of 25°C is crucial in order to obtain valuable results!!!

18. REFERENCES

References to AIM:


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

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Assay Procedure Summary

Antibody Coated Microtiter Strips

Reconstitute Biotin Labelled Antibody and Master Standard, prepare set of Standards

Dilute samples 500x

Add Standards and samples 100 µl

Incubate at RT for 1 hour / 300 rpm

Wash 3x

Prepare Wash Solution

Prepare Biotin Labeled Antibody Solution

Add Biotin Labelled Antibody solution 100 µl

Incubate at RT for 1 hour / 300 rpm

Wash 3x

Add Streptavidin-HRP Conjugate 100 µl

Incubate at RT for 30 min / 300 rpm

Wash 3x

Add Substrate Solution 100 µl

Incubate at RT for 10 min

Add Stop Solution 100 µl

Read absorbance and calculate results