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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 RD191481200R Human Azurocidin/HBP ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human azurocidin.

Features

- It is intended for research use only
- The total assay time is less than 3.5 hours
- The kit measures azurocidin in serum, plasma (EDTA, citrate, heparin) and urine.
- Assay format is 96 wells
- Standard is native 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.
Azurocidin, also known as cationic antimicrobial protein 37 kDa (CAP37) or heparin-binding protein (HBP) is an inactive homolog of serine proteinases residing in granulocytes. The ability to cleave peptide bond was lost due to replacement of two of the three residues from the conserved catalytic triad characteristic for serine proteinases. Azurocidin is a single polypeptide glycoprotein synthesized as a 251 amino-acid precursor which is subsequently processed by removal of 26 amino-acid residues from the N-terminus and three residues from the C-terminus. The mature polypeptide consists of 222 amino-acid residues with calculated molecular mass of 24 kDa. Azurocidin is an example of a protein which lost its primary proteolytic function in evolution, but gained another activity — it became an important mediator of inflammatory response. As a component of neutrophil azurophilic granules it participates in oxygen-independent killing mechanisms functioning in phagocytosing neutrophils. Azurocidin has a broad spectrum of antimicrobial activity, mainly against Gram-negative bacteria. Released extracellularly, azurocidin causes contraction of endothelial cells. Secreted azurocidin attracts monocytes and is responsible for their influx into inflammation sites. These properties make azurocidin a plausible target in new therapies directed against the harmful effects of inflammatory response [8]. Azurocidin is promising biomarker for identification of patients who are at risk in developing sepsis. Plasma HBP levels correlate with the severity of infection and, in particular, with the development of circulatory failure. There is close correlation between increased HBP plasma levels and the development of hypotension, organ failure, and septic shock [6]. Due to its antimicrobial features, elevated cerebrospinal fluid levels of HBP distinguish between patients with acute bacterial meningitis and patients with other central nervous system infections [5].

Areas of investigation:
Immune Response, Infection and Inflammation
Sepsis
4. TEST PRINCIPLE

In the BioVendor Human Azurocidin/HBP ELISA, standards and samples are incubated in microplate wells pre-coated with polyclonal anti-azurocidin antibody. After 60 minutes incubation and washing, biotin labelled polyclonal anti-azurocidin antibody is added and incubated for 60 minutes with captured azurocidin. After another washing, streptavidin-HRP conjugate is added. After 30 minutes 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 azurocidin. 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. 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 Conc. (100x)</td>
<td>concentrated</td>
<td>0.13 ml</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>50 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 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
- 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 desicant 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 Azurocidin 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 Human Azurocidin 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>0.625 ng/ml</td>
</tr>
</tbody>
</table>

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

Stability and storage:
Do not store the reconstituted Master Standard and/or diluted standard solutions.

Biotin Labelled Antibody Conc. (100x)
Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Concentrate (100x) to 99 parts Biotin-Ab Diluent. Example: 10 μl of Biotin Labelled Antibody Concentrate (100x) + 990 μl of Biotin-Ab Diluent for 1 strip (8 wells).

Stability and storage:
Do not store the diluted Biotin Labelled Antibody 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

The kit measures azurocidin in serum, plasma (EDTA, citrate, heparin) and urine samples.

Samples should be assayed immediately after collection or be stored at -20°C. 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 serum samples.

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

Recommended starting dilution for serum and heparin plasma is 50x. Dilute samples (serum, heparin plasma) 50x with Dilution Buffer just prior to the assay, e.g. 10 \( \mu \)l of sample + 490 \( \mu \)l of Dilution Buffer. Mix well (not to foam). Vortex is recommended.

Recommended starting dilution for EDTA and citrate plasma samples is 20x.

Recommended starting dilution for urine samples is 10x.

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.

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

1. Pipet **100 µl** of 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. Pipet **100 µl** 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 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against 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 paper towel.
10. Add **100 µl** of Substrate Solution. (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 below than 20°C). No shaking!
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 1:** 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 azurocidin 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 twice. 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</td>
<td>Sample 2</td>
<td>Sample 10</td>
<td>Sample 18</td>
<td>Sample 26</td>
<td>Sample 34</td>
</tr>
<tr>
<td>B</td>
<td>Standard 10</td>
<td>Sample 3</td>
<td>Sample 11</td>
<td>Sample 19</td>
<td>Sample 27</td>
<td>Sample 35</td>
</tr>
<tr>
<td>C</td>
<td>Standard 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>D</td>
<td>Standard 2.5</td>
<td>Sample 5</td>
<td>Sample 13</td>
<td>Sample 21</td>
<td>Sample 29</td>
<td>Sample 37</td>
</tr>
<tr>
<td>E</td>
<td>Standard 1.25</td>
<td>Sample 6</td>
<td>Sample 14</td>
<td>Sample 22</td>
<td>Sample 30</td>
<td>Sample 38</td>
</tr>
<tr>
<td>F</td>
<td>Standard 0.625</td>
<td>Sample 7</td>
<td>Sample 15</td>
<td>Sample 23</td>
<td>Sample 31</td>
<td>Sample 39</td>
</tr>
<tr>
<td>G</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>
<tr>
<td>H</td>
<td>Sample 1</td>
<td>Sample 9</td>
<td>Sample 17</td>
<td>Sample 25</td>
<td>Sample 33</td>
<td>Sample 41</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 azurocidin (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 (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. 2.5 ng/ml (from standard curve) x 50 (dilution factor) = 125 ng/ml.

Figure 2: Typical Standard Curve for Human Azurocidin/HBP ELISA.
13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human Azurocidin/HBP 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 azurocidin values in wells and is 0.06 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.

- **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>52.2</td>
<td>1.9</td>
<td>3.6</td>
</tr>
<tr>
<td>2</td>
<td>149.1</td>
<td>7.4</td>
<td>5.0</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>63.1</td>
<td>4.8</td>
<td>7.6</td>
</tr>
<tr>
<td>2</td>
<td>619.4</td>
<td>36.5</td>
<td>5.9</td>
</tr>
</tbody>
</table>
- **Spiking Recovery**
  Samples were spiked with different amounts of azurocidin 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>49.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>84.5</td>
<td>80.6</td>
<td>104.8</td>
</tr>
<tr>
<td></td>
<td>117.1</td>
<td>111.9</td>
<td>104.6</td>
</tr>
<tr>
<td></td>
<td>186.5</td>
<td>174.4</td>
<td>106.9</td>
</tr>
<tr>
<td>2</td>
<td>135.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>224.6</td>
<td>197.6</td>
<td>113.7</td>
</tr>
<tr>
<td></td>
<td>272.2</td>
<td>260.1</td>
<td>104.7</td>
</tr>
<tr>
<td></td>
<td>376.8</td>
<td>385.1</td>
<td>97.9</td>
</tr>
</tbody>
</table>

- **Linearity**
  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>211.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>111.2</td>
<td>105.7</td>
<td>105.2</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>55.4</td>
<td>52.9</td>
<td>104.7</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>26.2</td>
<td>26.4</td>
<td>99.0</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>600.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>273.4</td>
<td>300.4</td>
<td>91.0</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>138.5</td>
<td>150.2</td>
<td>92.2</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>78.0</td>
<td>75.1</td>
<td>103.8</td>
</tr>
</tbody>
</table>
Effect of sample matrix
EDTA, citrate and heparin plasmas were compared to respective serum samples from the same 10 individuals. We obtained very low correlation among serum and plasma samples. 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>363.6</td>
<td>14.7</td>
<td>38.6</td>
<td>241.3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>90.3</td>
<td>6.3</td>
<td>4.5</td>
<td>56.6</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>328.1</td>
<td>9.9</td>
<td>65.6</td>
<td>421.7</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>837.3</td>
<td>25.0</td>
<td>32.6</td>
<td>222.4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>112.7</td>
<td>11.5</td>
<td>17.1</td>
<td>38.5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>80.2</td>
<td>16.5</td>
<td>12.9</td>
<td>117.6</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>176.6</td>
<td>6.3</td>
<td>12.0</td>
<td>366.6</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>74.8</td>
<td>9.4</td>
<td>13.1</td>
<td>65.2</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>409.9</td>
<td>20.6</td>
<td>17.1</td>
<td>354.6</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>318.1</td>
<td>6.9</td>
<td>29.3</td>
<td>70.9</td>
<td></td>
</tr>
<tr>
<td>Mean (ng/ml)</td>
<td>279.2</td>
<td>12.7</td>
<td>24.3</td>
<td>195.5</td>
<td></td>
</tr>
<tr>
<td>Mean Plasma/Serum (%)</td>
<td>-</td>
<td>4.5</td>
<td>8.7</td>
<td>70.0</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3: HBP levels measured using Human Azurocidin/HBP ELISA in serum, EDTA, citrate and heparin plasma, respectively, from the same 10 individuals.
14. DEFINITION OF THE STANDARD

Human native azurocidin purified from human neutrophils is used as the standard.

15. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum samples from 150 unselected donors (86 men + 64 women) 21-65 years old were assayed with the BioVendor Human Azurocidin/HBP ELISA in our laboratory.

Presented results are multiplied by respective dilution factor

- Age dependent distribution of azurocidin

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (years)</th>
<th>n</th>
<th>azurocidin (ng/ml)</th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>Median</td>
<td>SD</td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>Men</td>
<td>21-29</td>
<td>18</td>
<td></td>
<td>143.2</td>
<td>105.7</td>
<td>103.5</td>
<td>39.1</td>
<td>452.7</td>
</tr>
<tr>
<td></td>
<td>30-39</td>
<td>24</td>
<td></td>
<td>173.3</td>
<td>160.9</td>
<td>113.0</td>
<td>31.5</td>
<td>455.5</td>
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<td>85.0</td>
<td>58.3</td>
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Figure 4: Human azurocidin concentration plotted against donor age and sex.

- **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 references ranges for azurocidin levels with the assay.
16. METHOD COMPARISON

The BioVendor Human Azurocidin/HBP ELISA was compared to another commercial immunoassay by measuring 52 samples. The following correlation graph was obtained:

![Method Comparison](image)

Figure 5: Method Comparison
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
• 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

High coefficient of variation (CV)
Possible explanation:
• Improper or inadequate washing
• Improper mixing Standards and samples
18. REFERENCES

References to azurocidin / HBP:


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

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<td>🧡 8 °C</td>
<td>Storage conditions</td>
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<tr>
<td>🏘️</td>
<td>Name and registered office of the manufacturer</td>
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**Antibody Coated Microtiter Plate**

- Reconstitute **Master Standard**, prepare set of **standards**

**Add standards and samples**

- 100 µl

- Incubate at RT for 1 hour / 300 RPM

**Add**

- **Biotin Labelled Antibody Solution**

- 100 µl

- Incubate at RT for 1 hour / 300 RPM

- **Add**

- **Streptavidin HRP Conjugate**

- 100 µl

- Incubate at RT for 30 min / 300 RPM

- **Add**

- **Substrate Solution**

- 100 µl

- Incubate at RT for 10 min

**Add**

- **Stop Solution**

- 100 µl

**Read absorbance and calculate results**

**Prepare Wash Solution**

- Wash 3x

- Incubate at RT for 1 hour / 300 RPM

**Prepare Biotin Labeled Antibody Solution**

- Wash 3x

- Incubate at RT for 30 min / 300 RPM

**Dilute samples:**

- serum, heparin plasma 50x,

- EDTA and citrate plasma 20x,

- urine 10x