## CONTENTS

1. INTENDED USE 3
2. STORAGE, EXPIRATION 3
3. INTRODUCTION 4
4. TEST PRINCIPLE 5
5. PRECAUTIONS 5
6. TECHNICAL HINTS 6
7. REAGENT SUPPLIED 6
8. MATERIAL REQUIRED BUT NOT SUPPLIED 7
9. PREPARATION OF REAGENTS 7
10. PREPARATION OF SAMPLES 9
11. ASSAY PROCEDURE 11
12. CALCULATIONS 13
13. PERFORMANCE CHARACTERISTICS 14
14. DEFINITION OF THE STANDARD 17
15. PRELIMINARY POPULATION DATA 17
16. METHOD COMPARISON 19
17. TROUBLESHOOTING AND FAQs 20
18. REFERENCES 21
19. EXPLANATION OF SYMBOLS 23

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 RD191367100R Human Angiotensinogen ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human angiotensinogen.

Features

- It is intended for research use only
- The total assay time is less than 2.5 hours
- The kit measures angiotensinogen in serum, plasma (EDTA, citrate, heparin), urine, cerebrospinal fluid (CSF), bronchoalveolar lavage fluid (BALF) and amniotic fluid
- 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.
Human angiotensinogen (AGT), also known as serpin peptidase inhibitor, clade A or member 8, is a 452-amino acid glycoprotein, a non-inhibitory member of the serpin family of proteinase inhibitors. Angiotensinogen is a component of the renin-angiotensin system (RAS), a hormone system that regulates blood pressure and fluid balance. AGT is the only defined substrate for renin which catalytically cleaves the 10 N-terminal amino acids of AGT to produce angiotensin I in response to lowered blood pressure. Angiotensin I is subsequently converted to the physiologically active peptide Angiotensin I through the removal of two C-terminal residues by the Angiotensin converting enzyme (ACE). Angiotensin I functions in the regulation of volume and mineral balance of body fluids. Both angiotensin I and angiotensin II can be further processed to generate angiotensin III, which stimulates aldosterone release, and angiotensin IV. AGT is the only precursor of all the angiotensin peptides and the cleavage of AGT by renin is the rate-limiting step of RAS. Besides systemic RAS, locally expressed components of RAS have been found in a number of tissues including the kidney, adrenal gland, heart, vasculature and nervous system. Localized RAS has a variety of functions, including local cardiovascular regulation, in association with or independently of the systemic RAS, as well as non-cardiovascular functions and influences on cell growth, differentiation, apoptosis, etc. [1,26]

Angiotensinogen is mainly produced in the liver and secreted in plasma, but is also synthesized in adipocytes, proximal tubule epithelial cells, and astrocytes. Among these tissues, only adipocytes have been shown to have an impact on plasma AGT level. In healthy individuals, AGT exists as a monomeric form (55-65 kDa) as well as a high molecular mass form (200-550 kDa) in plasma. In men and non-pregnant women with normal blood pressure, the high molecular mass form accounts for 3% of total circulating AGT. In the first half of pregnancy the total AGT increases 4-fold and the high molecular mass form increases 20-fold.[2,4]

In the AGT gene, M235T and T174M polymorphisms have been identified and they are associated with a predisposition to essential hypertension, atherosclerosis, obesity, ischemic stroke risk or preeclampsia.[6,14,15,16,17,18,19] Experimental research of local RAS and vascular remodeling revealed that augmented angiotensinogen secreted from apoptotic vascular endothelial cells is a vital mediator of vascular remodeling. A recent study of patients with IgA vasculitis revealed that higher serum AGT concentrations correlated with IgA vasculitis complicated by nephritis and could be used as a potential marker for the progression of IgA vasculitis.[22,23]

In a rat hypertension model, urinary angiotensinogen (UAGT) was identified which is produced and secreted by the kidney proximal tubules and does not come from plasma.[5] These findings show that UAGT is involved in the intrarenal RAS and reflects intrarenal angiotensin II activity associated with increased risk for deterioration of renal function in patients with chronic kidney disease such as diabetic nephropathy and membranous nephropathy as well as in patients with acute kidney injury.[7,8,9,10,11,12,20,21,24,25] In many studies, it has been reported that urinary AGT is increased in hypertensive patients. Treatment with RAS blockers suppresses UAGT levels which indicates that the efficacy of the RAS blockade to reduce the
intrarenal RAS activity can be assessed by measurement of UAGT.[3,13]

Areas of investigation:
Blood pressure regulation
Cardiovascular disease
Chronic renal failure
Renal disease

4. TEST PRINCIPLE

In the BioVendor Human Angiotensinogen ELISA, standards and samples are incubated in microplate wells pre-coated with polyclonal anti-human angiotensinogen antibody. After 60 minutes incubation and washing, polyclonal anti-human angiotensinogen antibody, conjugated with horseradish peroxidase (HRP) is added to the wells and incubated for 60 minutes with the captured angiotensinogen. 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 angiotensinogen. 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>Conjugate Solution Conc. (50x)</td>
<td>concentrated</td>
<td>0.26 ml</td>
</tr>
<tr>
<td>Master Standard</td>
<td>lyophilized</td>
<td>2 vials</td>
</tr>
<tr>
<td>Conjugate Diluent</td>
<td>ready to use</td>
<td>13 ml</td>
</tr>
<tr>
<td>Dilution Buffer Conc. (10x)</td>
<td>concentrated</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)
- Precise 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 shaking at 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 when stored at 2–8° C and protected from the moisture.

  **Conjugate 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 Angiotensinogen 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 angiotensinogen 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>0.32 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 diluted standard solutions.

Conjugate Solution Conc. (50x)
Prepare the working Conjugate Solution by adding 1 part Conjugate Solution Concentrate (50x) to 49 parts Conjugate Diluent. Example: 20 µl of Conjugate Solution Concentrate (50x) + 980 µl of Conjugate Diluent for 1 strip (8 wells).
Stability and storage:
Opened Conjugate Solution Concentrate (50x) is stable 3 months when stored at 2–8°C.
Do not store the diluted Conjugate Solution.

Dilution Buffer Conc. (10x)
Dilute the Dilution Buffer Concentrate (10x) ten-fold in distilled water to prepare the 1x working solution, e.g. 20 ml of Dilution Buffer Concentrate (10x) + 180 ml of distilled water for use of all 96-wells.
It is recommended to prepare the Dilution Buffer working solution just prior to the test, using only the amount of the Dilution Buffer Concentrate (10x) which is needed for the test.
Stability and storage:
The diluted Dilution Buffer is stable 1 week when stored at 2–8°C. Opened Dilution Buffer Concentrate (10x) is stable 3 months when stored at 2–8°C.
Wash Solution Conc. (10x)

Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare the 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 angiotensinogen in serum, plasma (EDTA, citrate, heparin), urine, cerebrospinal fluid (CSF), bronchoalveolar lavage fluid (BALF) and amniotic fluid.

Samples can be assayed immediately after collection or should be stored frozen. Mix thawed samples thoroughly 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 prior to batch measurement.

Recommended starting dilution for serum and plasma is 10 000x.

Dilute samples (serum, plasma) 10 000x with the Dilution Buffer just prior to the assay in two steps as follows:

**Dilution A (100x):**
Add 10 µl of sample into 990 µl of Dilution Buffer. **Mix well** (not to foam). Vortex is recommended.

**Dilution B (100x):**
Add 10 µl of Dilution A into 990 µl of Dilution Buffer. **Mix well** (not to foam). Vortex is recommended.

Recommended starting dilution for urine and BALF samples is 3x.
Recommended starting dilution for CSF and amniotic fluid samples is 200x.

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 angiotensinogen.
**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 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 of 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 15 minutes at room temperature. 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 9.

Note 1: 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 angiotensinogen 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 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</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</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 1.25</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 0.63</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 0.32</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 angiotensinogen (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 they have been diluted prior to the assay, e.g. 5 ng/ml (from standard curve) x 10 000 (dilution factor) = 50 000 ng/ml = 50 μg/ml.

![Human Angiotensinogen ELISA Standard Curve](image)

*Figure 2: Typical standard curve for Human Angiotensinogen ELISA.*
13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human Angiotensinogen 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: $A_{\text{blank}} + 3x\text{SD}_{\text{blank}}$) is calculated from the real human angiotensinogen values in wells and is 25 pg/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.

- Specifity
The antibodies used in this ELISA are specific for human angiotensinogen with no detectable crossreactivities to human angiotensin I and angiotensin II.

Presented results are multiplied by respective dilution factor

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean ($\mu$g/ml)</th>
<th>SD ($\mu$g/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>40.9</td>
<td>2.4</td>
<td>5.8</td>
</tr>
<tr>
<td>Serum 2</td>
<td>48.9</td>
<td>2.0</td>
<td>4.0</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean ($\mu$g/ml)</th>
<th>SD ($\mu$g/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>40.7</td>
<td>2.6</td>
<td>6.3</td>
</tr>
<tr>
<td>Serum 2</td>
<td>86.4</td>
<td>4.9</td>
<td>5.7</td>
</tr>
</tbody>
</table>
- **Spiking Recovery**
  Serum samples were spiked with different amounts of human angiotensinogen and assayed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Observed (µg/ml)</th>
<th>Expected (µg/ml)</th>
<th>Recovery O/E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>42.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>66.8</td>
<td>67.6</td>
<td>98.8</td>
</tr>
<tr>
<td></td>
<td>92.0</td>
<td>92.6</td>
<td>99.3</td>
</tr>
<tr>
<td></td>
<td>135.0</td>
<td>142.6</td>
<td>94.6</td>
</tr>
<tr>
<td>Serum 2</td>
<td>31.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>85.1</td>
<td>81.3</td>
<td>101.7</td>
</tr>
<tr>
<td></td>
<td>54.1</td>
<td>56.3</td>
<td>96.2</td>
</tr>
<tr>
<td></td>
<td>44.5</td>
<td>43.8</td>
<td>104.7</td>
</tr>
</tbody>
</table>

- **Linearity**
  Serum, plasma and urine samples were serially diluted with Dilution Buffer and assayed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Observed (µg/ml)</th>
<th>Expected (µg/ml)</th>
<th>Recovery O/E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>-</td>
<td>110.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>61.2</td>
<td>55.2</td>
<td>110.8</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>30.2</td>
<td>27.6</td>
<td>109.2</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>15.9</td>
<td>13.8</td>
<td>115.1</td>
</tr>
<tr>
<td>Serum 2</td>
<td>-</td>
<td>99.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>54.3</td>
<td>49.9</td>
<td>108.7</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>27.1</td>
<td>25.0</td>
<td>108.7</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>14.1</td>
<td>12.5</td>
<td>113.0</td>
</tr>
</tbody>
</table>

<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>Urine</td>
<td>-</td>
<td>10.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>5.3</td>
<td>5.0</td>
<td>105.6</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>2.5</td>
<td>2.5</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>1.1</td>
<td>1.3</td>
<td>91.4</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 (μg/ml)</th>
<th>Plasma (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum</td>
<td>EDTA</td>
</tr>
<tr>
<td>1</td>
<td>26.5</td>
<td>27.2</td>
</tr>
<tr>
<td>2</td>
<td>38.8</td>
<td>41.6</td>
</tr>
<tr>
<td>3</td>
<td>25.6</td>
<td>26.1</td>
</tr>
<tr>
<td>4</td>
<td>30.1</td>
<td>31.4</td>
</tr>
<tr>
<td>5</td>
<td>33.8</td>
<td>33.9</td>
</tr>
<tr>
<td>6</td>
<td>47.1</td>
<td>52.3</td>
</tr>
<tr>
<td>7</td>
<td>18.5</td>
<td>18.4</td>
</tr>
<tr>
<td>8</td>
<td>52.7</td>
<td>45.6</td>
</tr>
<tr>
<td>9</td>
<td>41.8</td>
<td>43.9</td>
</tr>
<tr>
<td>10</td>
<td>35.1</td>
<td>35.4</td>
</tr>
</tbody>
</table>

Mean (μg/ml) 35.0 | 35.6 | 30.7 | 38.8
Mean Plasma/Serum (%) | 101.6 | 87.8 | 110.8
Coefficient of determination R² | 0.91 | 0.91 | 0.90

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

Recombinant human angiotensinogen is used as the standard. The recombinant human angiotensinogen produced in HEK293 cells is a 51.0 kDa protein containing 452 amino acid residues of human angiotensinogen and 10 extra AA.

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 Angiotensinogen ELISA in our laboratory.

- **Age dependent distribution of angiotensinogen**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (years)</th>
<th>n</th>
<th>Angiotensinogen (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Men</td>
<td>21-29</td>
<td>18</td>
<td>38.8</td>
</tr>
<tr>
<td></td>
<td>30-39</td>
<td>26</td>
<td>46.3</td>
</tr>
<tr>
<td></td>
<td>40-49</td>
<td>31</td>
<td>43.6</td>
</tr>
<tr>
<td></td>
<td>50-65</td>
<td>14</td>
<td>43.7</td>
</tr>
<tr>
<td>Women</td>
<td>21-29</td>
<td>12</td>
<td>103.1</td>
</tr>
<tr>
<td></td>
<td>30-39</td>
<td>26</td>
<td>75.7</td>
</tr>
<tr>
<td></td>
<td>40-49</td>
<td>20</td>
<td>58.1</td>
</tr>
<tr>
<td></td>
<td>50-61</td>
<td>8</td>
<td>52.2</td>
</tr>
</tbody>
</table>
Figure 4: Human angiotensinogen 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 sample in the assay. Each laboratory should establish its own normal and pathological reference ranges for angiotensinogen levels with the assay.
16. METHOD COMPARISON

The BioVendor Human Angiotensinogen ELISA was compared to another commercial immunoassay by measuring 36 serum samples. The following correlation graph was obtained:

![Method Comparison - serum samples](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
- Incubation temperature over 30°C

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


For more references on this product see our WebPages at www.biovendor.com
### 19. 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>🦠</td>
<td>Potential biological hazard</td>
</tr>
<tr>
<td>⌛️</td>
<td>Expiry date</td>
</tr>
<tr>
<td>🔄 2 °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

- Prepare Dilution Buffer
- Reconstitute Master Standard, and prepare set of standards
- Prepare Wash Solution
- Prepare Conjugate Solution
- Antibody Coated Microtiter Plate
- Add standards, and samples 100 μl
- Incubate at RT for 1 hour / 300 rpm
- Wash 3x
- Add Conjugate Solution 100 μl
- Incubate at RT for 1 hour / 300 rpm
- Wash 3x
- Add Substrate Solution 100 μl
- Incubate at RT for 15 min
- Add Stop Solution 100 μl
- Read absorbance and calculate results
- Dilute samples
BioVendor – Laboratorní medicína 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