HUMAN NT-PROBNP ELISA

Product Data Sheet

Cat. No.: RD191486200R

For Research Use Only
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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 RD191486200R Human NT-proBNP ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human N-terminal pro-B-type natriuretic peptide (NT-proBNP).

Features

- **It is intended for research use only**
- The assay time is less than 3.5 hours
- The kit measures NT-proBNP in serum, plasma (EDTA, citrate, heparin), urine and cerebrospinal fluid
- Assay format is 96 wells
- Quality Controls are human serum based
- 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.
Natriuretic peptides (NPs) are synthesized and secreted through certain mechanisms by cardiomyocytes, fibroblasts, endotheliocytes, immune cells (neutrophils, T-cells and macrophages) and immature cells (embryonic stem cells, muscle satellite cells and cardiac precursor cells). They are mainly produced by cardiovascular, brain and renal tissues in response to wall stretch and other causes. NPs provide natriuresis, diuresis, vasodilation, antiproliferation, antihypertrophy, antifibrosis and other cardiometabolic protection. NPs represent body’s own antihypertensive system, and provide compensatory protection to counterbalance vasoconstrictor, mitogenic, sodium-retaining hormones, released by renin-angiotensin-aldosterone system (RAAS) and sympathetic nervous system (SNS). NPs play central roles in the regulation of heart failure (HF), and are inactivated through not only NP receptor-C, but also neutral endopeptidase (NEP), dipeptidyl peptidase-4 and insulin degrading enzyme. Both BNP and N-terminal proBNP are useful biomarkers not only for diagnosis and severity of HF, but also to guide the therapy and predict the prognosis in patients with HF[1].

NT-proBNP (N-terminal pro-B-type natriuretic peptide) is an inactive and stable amino acid fragment co-secreted alongside the neuroendocrine peptide BNP from the ventricular cardiac myocytes. It is released in response to left ventricular strain or ischaemia and has been found to be an important biomarker for left ventricular systolic dysfunction and left ventricular stress in the general population [5]. NT-proBNP is predominantly cleared by the kidneys, and has a longer half-life than BNP (1-2 h vs. 20 min) leading to higher circulating levels and greater stability. Because NT-proBNP is metabolized in the kidneys, the serum level of NT-proBNP can be increased in patients with diseases that damage renal function [4]. Elevated levels of NT-pro BNP were described in patients with different cardiovascular pathologies such as heart failure, myocardial infarction and pulmonary embolism, acute or chronic cor pulmonale, acute dyspnea, and also in patients with non-cardiac pathologies, e.g. anemia, chronic renal failure or sepsis [3,6,7,8,9,10].

NT-proBNP may predict heart failure in children with sepsis. In addition, it provides an important clinical reference for the diagnosis of heart failure in pediatric patients with sepsis, and enables monitoring septic children for cardiac involvement [2].

In recent years, NT-proBNP has been found to be an excellent predictor of cardiovascular risk and mortality in patients with diabetes and can also be used for prognostic applications in patients with acute coronary syndrome [11,12]. Measuring NT-proBNP in primary care patients with suspected heart failure identified those with substantial increases in the risk of hospitalization for cardiovascular disease and death. Thus, NT-pro BNP is an ideal biomarker for heart failure in the primary care setting.

Areas of investigation
Cardiovascular disease
Renal disease
Diabetology
Neurodegenerative disease
4. TEST PRINCIPLE

In the BioVendor Human NT-proBNP ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with polyclonal anti-human NT-proBNP antibody. After 60 minutes incubation and washing, biotin labelled polyclonal anti-human NT-proBNP antibody is added and incubated for 60 minutes with captured NT-proBNP. 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 NT-proBNP. 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 contains components of human origin. These materials were found non-reactive for HBsAg, HCV antibody and for HIV 1/2 antigen and antibody. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents
- 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 been 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>Quality Control HIGH</td>
<td>lyophilized</td>
<td>2 vials</td>
</tr>
<tr>
<td>Quality Control LOW</td>
<td>lyophilized</td>
<td>2 vials</td>
</tr>
<tr>
<td>Dilution Buffer</td>
<td>ready to use</td>
<td>2x 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 microtitrate 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.

  Streptavidin-HRP Conjugate
  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 NT-proBNP 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 NT-proBNP in the stock solution is 320 pg/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>320 pg/ml</td>
</tr>
<tr>
<td>250 µl of stock</td>
<td>250 µl</td>
<td>160 pg/ml</td>
</tr>
<tr>
<td>250 µl of 160 pg/ml</td>
<td>250 µl</td>
<td>80 pg/ml</td>
</tr>
<tr>
<td>250 µl of 80 pg/ml</td>
<td>250 µl</td>
<td>40 pg/ml</td>
</tr>
<tr>
<td>250 µl of 40 pg/ml</td>
<td>250 µl</td>
<td>20 pg/ml</td>
</tr>
<tr>
<td>250 µl of 20 pg/ml</td>
<td>250 µl</td>
<td>10 pg/ml</td>
</tr>
<tr>
<td>250 µl of 10 pg/ml</td>
<td>250 µl</td>
<td>5 pg/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.

**Quality Controls HIGH, LOW**

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution and for current Quality Control concentration!!!

Reconstitute each Quality Control (HIGH and LOW) with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).

Reconstituted Quality Controls are ready to use, do not dilute them.

Stability and storage:
Do not store the reconstituted Quality Controls.

*Note:*
Concentration of analyte in Quality Controls need not be anyhow associated with normal and/or pathological concentrations in serum or another body fluid. Quality Controls serve just for control that the kit works in accordance with PDS and CoA and that ELISA test was carried out properly.

**Biotin Labelled Antibody Conc. (100x)**

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

Stability and storage:
Opened Biotin Labelled Antibody Conc. (100x) is stable 3 months when stored at 2-8°C.

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 NT-proBNP in serum, plasma (EDTA, citrate, heparin), urine and cerebrospinal fluid (CSF).

Samples should be assayed immediately after collection, or should be stored frozen. 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 researched prior to batch measurement, with respect to large variability in NT-proBNP serum levels (healthy individuals exhibit low NT-proBNP levels while several cardiac as well as non-cardiac pathologies are known to increase NT-proBNP levels markedly). Recommended starting dilution for samples from healthy donors is 3-fold, recommended starting dilution for samples from patients (suspected of) having such a disease is 50-fold.

Recommended starting dilution for serum, plasma, urine and CSF samples is 3x:
Dilute samples 3x with Dilution Buffer just prior to the assay, e.g. 50 μl of sample + 100 μl of Dilution Buffer for singlets, or preferably 100 μl of sample + 200 μl of Dilution Buffer for duplicates. Mix well (not to foam). Vortex is recommended.

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

See Chapter 13 for stability of serum and plasma samples when stored at 2-8°C, effect of freezing/thawing and effect of sample matrix (serum/plasma) on the concentration of human NT-proBNP.

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, Quality Controls, 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 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 below 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 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 NT-proBNP 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 320</td>
<td>QC HIGH</td>
<td>Sample 7</td>
<td>Sample 15</td>
<td>Sample 23</td>
<td>Sample 31</td>
</tr>
<tr>
<td>B</td>
<td>Standard 160</td>
<td>QC LOW</td>
<td>Sample 8</td>
<td>Sample 16</td>
<td>Sample 24</td>
<td>Sample 32</td>
</tr>
<tr>
<td>C</td>
<td>Standard 80</td>
<td>Sample 1</td>
<td>Sample 9</td>
<td>Sample 17</td>
<td>Sample 22</td>
<td>Sample 33</td>
</tr>
<tr>
<td>D</td>
<td>Standard 40</td>
<td>Sample 2</td>
<td>Sample 10</td>
<td>Sample 18</td>
<td>Sample 26</td>
<td>Sample 34</td>
</tr>
<tr>
<td>E</td>
<td>Standard 20</td>
<td>Sample 3</td>
<td>Sample 11</td>
<td>Sample 19</td>
<td>Sample 27</td>
<td>Sample 35</td>
</tr>
<tr>
<td>F</td>
<td>Standard 10</td>
<td>Sample 4</td>
<td>Sample 12</td>
<td>Sample 20</td>
<td>Sample 28</td>
<td>Sample 36</td>
</tr>
<tr>
<td>G</td>
<td>Standard 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>H</td>
<td>Blank</td>
<td>Sample 6</td>
<td>Sample 14</td>
<td>Sample 22</td>
<td>Sample 30</td>
<td>Sample 38</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 NT-proBNP (pg/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. 20 pg/ml (from standard curve) x 3 (dilution factor) = 60 pg/ml

Figure 2: Typical Standard Curve for Human NT-proBNP ELISA.
13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human NT-proBNP 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 NT-proBNP values in wells and is 1 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.

- Specificity
  The antibodies used in this ELISA are specific for human NT-proBNP with no detectable crossreactivity to human NT-proANP.
  We observed no interference of hemoglobin (1.0 mg/ml), bilirubin (170 $\mu$mol/l) and triglycerides (5.0 mmol/l) on the measurement of NT-proBNP.
  Sera of several mammalian species were measured in the assay. See results below.
  For details please contact us at info@biovendor.com.

<table>
<thead>
<tr>
<th>Mammalian serum sample</th>
<th>Observed crossreactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine</td>
<td>no</td>
</tr>
<tr>
<td>Cat</td>
<td>no</td>
</tr>
<tr>
<td>Dog</td>
<td>no</td>
</tr>
<tr>
<td>Goat</td>
<td>no</td>
</tr>
<tr>
<td>Hamster</td>
<td>no</td>
</tr>
<tr>
<td>Horse</td>
<td>no</td>
</tr>
<tr>
<td>Monkey</td>
<td>no</td>
</tr>
<tr>
<td>Mouse</td>
<td>no</td>
</tr>
<tr>
<td>Pig</td>
<td>no</td>
</tr>
<tr>
<td>Rabbit</td>
<td>no</td>
</tr>
<tr>
<td>Rat</td>
<td>no</td>
</tr>
<tr>
<td>Sheep</td>
<td>no</td>
</tr>
</tbody>
</table>
Presented results are multiplied by respective dilution factor

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (pg/ml)</th>
<th>SD (pg/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>154.2</td>
<td>9.7</td>
<td>6.3</td>
</tr>
<tr>
<td>Serum 2</td>
<td>52.9</td>
<td>3.1</td>
<td>5.9</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (pg/ml)</th>
<th>SD (pg/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>71.0</td>
<td>4.8</td>
<td>6.8</td>
</tr>
<tr>
<td>Serum 2</td>
<td>140.8</td>
<td>8.4</td>
<td>6.0</td>
</tr>
</tbody>
</table>

- **Spiking Recovery**
  Samples were spiked with different amounts of human NT-proBNP and assayed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Observed (pg/ml)</th>
<th>Expected (pg/ml)</th>
<th>Recovery O/E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>82.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>145.4</td>
<td>142.1</td>
<td>102.3</td>
</tr>
<tr>
<td></td>
<td>199.3</td>
<td>202.1</td>
<td>98.6</td>
</tr>
<tr>
<td></td>
<td>318.6</td>
<td>322.1</td>
<td>98.9</td>
</tr>
<tr>
<td>Serum 2</td>
<td>38.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>95.9</td>
<td>98.9</td>
<td>97.0</td>
</tr>
<tr>
<td></td>
<td>150.6</td>
<td>158.9</td>
<td>94.7</td>
</tr>
<tr>
<td></td>
<td>269.6</td>
<td>278.9</td>
<td>96.6</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 (pg/ml)</th>
<th>Expected (pg/ml)</th>
<th>Recovery O/E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>-</td>
<td>220.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>100.2</td>
<td>110.1</td>
<td>91.0</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>46.9</td>
<td>55.0</td>
<td>85.3</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>22.7</td>
<td>27.5</td>
<td>82.6</td>
</tr>
<tr>
<td>Serum 2</td>
<td>-</td>
<td>167.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>79.7</td>
<td>83.7</td>
<td>95.2</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>40.5</td>
<td>41.9</td>
<td>96.8</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>19.2</td>
<td>20.9</td>
<td>91.6</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 (pg/ml)</th>
<th>Plasma (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EDTA</td>
</tr>
<tr>
<td>1</td>
<td>62.2</td>
<td>64.2</td>
</tr>
<tr>
<td>2</td>
<td>96.4</td>
<td>103.7</td>
</tr>
<tr>
<td>3</td>
<td>35.2</td>
<td>42.6</td>
</tr>
<tr>
<td>4</td>
<td>92.3</td>
<td>87.6</td>
</tr>
<tr>
<td>5</td>
<td>20.4</td>
<td>23.9</td>
</tr>
<tr>
<td>6</td>
<td>28.0</td>
<td>29.7</td>
</tr>
<tr>
<td>7</td>
<td>32.5</td>
<td>36.2</td>
</tr>
<tr>
<td>8</td>
<td>27.4</td>
<td>33.1</td>
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<tr>
<td>9</td>
<td>34.3</td>
<td>39.4</td>
</tr>
<tr>
<td>10</td>
<td>25.0</td>
<td>25.2</td>
</tr>
<tr>
<td><strong>Mean (pg/ml)</strong></td>
<td><strong>45.4</strong></td>
<td><strong>48.6</strong></td>
</tr>
<tr>
<td><strong>Mean Plasma/Serum (%)</strong></td>
<td>107.0</td>
<td>94.2</td>
</tr>
<tr>
<td><strong>Coefficient of determination R²</strong></td>
<td>0.98</td>
<td>0.98</td>
</tr>
</tbody>
</table>
Figure 3: NT-proBNP levels measured using Human NT-proBNP ELISA in serum, EDTA, citrate and heparin plasma, respectively, from the same 10 individuals.

- **Stability of samples stored at 2-8°C**
  Prolonged storage of samples at 2-8°C can lead to a decline in measured concentration of NT-proBNP. Therefore, we recommend to store the samples at -20°C, or preferably at -70°C for long-term storage. To avoid microbial contamination, samples were treated with epsilon-aminocaproic acid and thimerosal, resulting in the final concentration of 0.03% and 0.01%, respectively.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Incubation Temp, Period</th>
<th>Serum (pg/ml)</th>
<th>Plasma (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Serum EDTA</td>
<td>Serum Citrate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-20°C</td>
<td>33.0</td>
<td>36.6</td>
</tr>
<tr>
<td></td>
<td>2-8°C, 1 day</td>
<td>40.7</td>
<td>40.8</td>
</tr>
<tr>
<td></td>
<td>2-8°C, 7 days</td>
<td>25.3</td>
<td>34.2</td>
</tr>
<tr>
<td>2</td>
<td>-20°C</td>
<td>48.8</td>
<td>48.3</td>
</tr>
<tr>
<td></td>
<td>2-8°C, 1 day</td>
<td>35.0</td>
<td>40.0</td>
</tr>
<tr>
<td></td>
<td>2-8°C, 7 days</td>
<td>23.6</td>
<td>40.6</td>
</tr>
<tr>
<td>3</td>
<td>-20°C</td>
<td>31.6</td>
<td>34.7</td>
</tr>
<tr>
<td></td>
<td>2-8°C, 1 day</td>
<td>41.1</td>
<td>39.7</td>
</tr>
<tr>
<td></td>
<td>2-8°C, 7 days</td>
<td>22.0</td>
<td>31.9</td>
</tr>
</tbody>
</table>

- **Effect of Freezing/Thawing**
  No decline was observed in concentration of human NT-proBNP in serum and plasma samples after repeated (5x) freeze/thaw cycles. However, it is recommended to avoid unnecessary repeated freezing/thawing of the samples.
14. DEFINITION OF THE STANDARD

Recombinant human NT-proBNP is used as the standard. The recombinant human NT-proBNP produced in E. coli is a 9.4 kDa protein containing 75 amino acid residues of human NT-proBNP and 8 extra amino acids.

15. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum samples from 151 unselected donors (83 men + 68 women) 21 – 65 years old were assayed with the BioVendor Human NT-proBNP ELISA in our laboratory.

<table>
<thead>
<tr>
<th>Number of f/t cycles</th>
<th>Serum (pg/ml)</th>
<th>Plasma (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EDTA</td>
</tr>
<tr>
<td>1</td>
<td>1x</td>
<td>95.8</td>
</tr>
<tr>
<td></td>
<td>3x</td>
<td>103.1</td>
</tr>
<tr>
<td></td>
<td>5x</td>
<td>92.6</td>
</tr>
<tr>
<td>2</td>
<td>1x</td>
<td>45.1</td>
</tr>
<tr>
<td></td>
<td>3x</td>
<td>42.7</td>
</tr>
<tr>
<td></td>
<td>5x</td>
<td>49.7</td>
</tr>
<tr>
<td>3</td>
<td>1x</td>
<td>36.7</td>
</tr>
<tr>
<td></td>
<td>3x</td>
<td>42.5</td>
</tr>
<tr>
<td></td>
<td>5x</td>
<td>30.9</td>
</tr>
</tbody>
</table>
Figure 4: Human NT-proBNP 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 reference ranges for human NT-proBNP protein levels with the assay.

16. **METHOD COMPARISON**

The BioVendor Human NT-proBNP ELISA was compared to a commercial ECLIA immunoassay by measuring 29 serum and plasma samples. The following correlation graph was obtained.
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 and samples
18. REFERENCES


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

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tr>
<td>Cont.</td>
<td>Content</td>
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<tr>
<td><img src="image" alt="Attention symbol" /></td>
<td>Attention, see instructions for use</td>
</tr>
<tr>
<td><img src="image" alt="Potential biological hazard symbol" /></td>
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</tr>
<tr>
<td><img src="image" alt="Expiry date symbol" /></td>
<td>Expiry date</td>
</tr>
<tr>
<td><img src="image" alt="Storage conditions symbol" /></td>
<td>Storage conditions</td>
</tr>
<tr>
<td><img src="image" alt="Registered office symbol" /></td>
<td>Name and registered office of the manufacturer</td>
</tr>
</tbody>
</table>
Antibody Coated Microtiter Plate

Reconstitute QCs and Master Standard, prepare set of standards

Add standards, QCs and samples 100 µl

Prepare Wash Solution

Wash 3x

Add Biotin Labelled Antibody solution 100 µl

Prepare Biotin Labeled Antibody solution

Add Streptavidin HRP Conjugate 100 µl

Wash 3x

Add Substrate Solution 100 µl

Add Stop Solution 100 µl

Incubate at RT for 1 hour / 300 rpm

Incubate at RT for 1 hour / 300 rpm

Incubate at RT for 30 min / 300 rpm

Incubate at RT for 10 min

Read absorbance and calculate results
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