HUMAN INTERLEUKIN-6 ELISA

Product Data Sheet

Cat. No.: RD194015200R

European Union: IVD

Rest of the world: For research use only!
1. INTENDED USE

The RD194015200R Human Interleukin-6 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human interleukin-6 (IL-6).

Features

- **European Union: for in vitro diagnostic use**
  - Rest of the world: for research use only!
- The assay time is less than 3.5 hours
- The kit measures IL-6 in serum, plasma (EDTA, citrate, heparin), urine, bronchoalveolar lavage fluid (BALF), cerebrospinal fluid (CSF) and amniotic 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.
3. INTRODUCTION

Interleukin 6 (IL-6), also named B-cell stimulatory factor (BSF-2), hepatocyte-stimulating factor (HSF), hybridoma growth factor (HGF) or interferon-β2 (IFN-β2), is a 26-kDa glycoprotein composed of 184 amino acids. It belongs to a large family of cytokines, which all share the four-helical protein topology. IL-6 is a cytokine with multiple pleiotropic effects on inflammation, immune response, hematopoiesis and oncogenesis.\[1\]

IL-6 pathways for induction of intracellular signaling comprise classic signaling and trans-signaling. Classic signaling pathway represents anti-inflammatory activities of IL-6 primarily in hepatocytes and lymphocytes through direct binding of IL-6 with non-signaling membrane-bound IL-6 α-receptor (mIL-6R) associated with membrane-bound glycoprotein 130 (gp130) which mediates signal transduction. In this pathway, IL-6 is mainly responsible for the beneficial regenerative and antibacterial processes. Trans-signaling pathway represents pro-inflammatory activities of IL-6 through binding of IL-6 with soluble IL-6 receptor (sIL-6R) and subsequent binding of this heterodimer with membrane-bound gp130. This pathway is present in all cells of the body since all the cells express the gp130 protein and sIL-6R occurs naturally in body fluids. On the other hand, mIL-6R expression is restricted to several cell types such as hepatocytes and some leukocytes.\[1,2\]

IL-6 is expressed in many types of cells, such as adipocytes, fibroblasts, osteoblasts, endothelial cells, neurons, lymphocytes, monocytes, neutrophils etc. and its expression is dependent on cell activation. Circulating IL-6 levels in healthy people are very low, about 1 pg/ml, but during inflammation, IL-6 levels increase rapidly. During the initial stage of inflammation at a local lesion IL-6 is synthesized, moves to the liver through the bloodstream and is followed by the rapid induction of an wide range of acute phase proteins such as C-reactive protein (CRP), serum amyloid A, haptoglobin, and α1-antichymotrypsin. Also, in the hypothalamus IL-6 induces fever. On the other hand, IL-6 reduces production of fibronectin, albumin and transferrin. In bone marrow, IL-6 promotes megakaryocyte maturation, leading to the release of platelets. These changes in acute phase protein levels and red blood cell and platelet counts are used for evaluation of inflammation severity in routine clinical laboratory examinations. Furthermore, IL-6 promotes specific differentiation of naïve CD4+ T cells, thus playing an important role in linking innate to acquired immune response. IL-6 also represents a warning signal upon tissue damage or cell death in noninfectious inflammation such as burn or trauma. IL-6 expression is strictly controlled by transcriptional and posttranscriptional mechanisms; dysregulated continual synthesis of IL-6 exerts negative effects on chronic inflammation and autoimmunity.\[5,6,7\]

Increased levels of IL-6 have been found in patients with atherosclerotic cardiovascular disease, insulin resistance, advanced stage of cancer, atopic asthma, rheumatoid arthritis, and circulating micrometastasis etc.\[4,8,10,11,12,14,15,16,17\]

Patients with metabolic syndrome have significantly greater serum IL-6 and TNF-α levels than controls, supporting the hypothesis that inflammation plays an important role in the immunopathogenesis of the disease.\[9\] Chronically elevated IL-6 levels were detected in patients with diabetes mellitus type 2 and obesity.\[18\] In critically ill patients, serum levels of IL-6 reflect severity of organ dysfunction more accurately compared to procalcitonin and CRP.\[15\]
Many studies have demonstrated that deregulated overexpression of IL-6 is associated with tumor progression through inhibition of cancer cell apoptosis, stimulation of angiogenesis, and drug resistance. Increased serum IL-6 levels in these patients are associated with advanced tumor stages. Therefore, blocking IL-6 signaling is a potential therapeutic strategy for cancer characterized by pathological IL-6 production.[3]

Areas of investigation
Atherosclerosis
Cardiovascular disease
Immune response, Infection and Inflammation
Metabolic syndrome
Oncology

4. TEST PRINCIPLE

In the BioVendor Human Interleukin-6 ELISA, standards and samples are incubated in microplate wells pre-coated with monoclonal anti-human IL-6 antibody. After 60 minutes incubation and washing, biotin labelled monoclonal anti-human IL-6 antibody is added and incubated for 60 minutes with captured IL-6. 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 IL-6. 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
- This kit may contain components of 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 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 IL-6 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 IL-6 in the stock solution is 80 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>80 pg/ml</td>
</tr>
<tr>
<td>250 µl of stock</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>
<tr>
<td>250 µl of 5 pg/ml</td>
<td>250 µl</td>
<td>2.5 pg/ml</td>
</tr>
<tr>
<td>250 µl of 2.5 pg/ml</td>
<td>250 µl</td>
<td>1.25 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 IL-6 in serum, plasma (EDTA, citrate, heparin), urine, bronchoalveolar lavage fluid (BALF), cerebrospinal fluid (CSF) and amniotic fluid.

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 researcher prior to batch measurement.

Recommended starting dilution for serum and plasma 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.

Recommended starting dilution for urine, bronchoalveolar lavage fluid, cerebrospinal fluid is 3x.
Recommended starting dilution for amniotic fluid is 50x.

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 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 IL-6.

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 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 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 IL-6 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 80</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 40</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 20</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 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>E</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>F</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>G</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>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 IL-6 (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. 25 pg/ml (from standard curve) x 3 (dilution factor) = 75 pg/ml.

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**Figure 2: Typical Standard Curve for Human IL-6 ELISA.**
13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human Interleukin-6 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: $A_{\text{blank}} + 3\times SD_{\text{blank}}$) is calculated from the real IL-6 values in wells and is 0.32 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.

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>43.2</td>
<td>2.2</td>
<td>5.1</td>
</tr>
<tr>
<td>Serum 2</td>
<td>73.3</td>
<td>3.0</td>
<td>4.2</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>36.8</td>
<td>1.7</td>
<td>4.7</td>
</tr>
<tr>
<td>Serum 2</td>
<td>140.8</td>
<td>7.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>
- **Spiking Recovery**
  Samples were spiked with different amounts of human IL-6 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>20.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>35.5</td>
<td>35.3</td>
<td>100.5</td>
</tr>
<tr>
<td></td>
<td>49.1</td>
<td>50.3</td>
<td>97.6</td>
</tr>
<tr>
<td></td>
<td>77.4</td>
<td>80.3</td>
<td>96.3</td>
</tr>
<tr>
<td>Serum 2</td>
<td>21.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>38.4</td>
<td>36.3</td>
<td>105.9</td>
</tr>
<tr>
<td></td>
<td>53.7</td>
<td>51.3</td>
<td>104.7</td>
</tr>
<tr>
<td></td>
<td>83.2</td>
<td>81.3</td>
<td>102.5</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>156.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>72.7</td>
<td>78.3</td>
<td>92.8</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>35.5</td>
<td>39.1</td>
<td>90.7</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>17.3</td>
<td>19.6</td>
<td>88.6</td>
</tr>
<tr>
<td>Serum 2</td>
<td>-</td>
<td>344.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>161.3</td>
<td>172.1</td>
<td>93.7</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>77.3</td>
<td>86.1</td>
<td>89.8</td>
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<tr>
<td></td>
<td>8x</td>
<td>39.4</td>
<td>43.0</td>
<td>91.6</td>
</tr>
<tr>
<td>urine</td>
<td>-</td>
<td>51.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>24.0</td>
<td>25.9</td>
<td>92.5</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>11.3</td>
<td>13.0</td>
<td>87.4</td>
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<td></td>
<td>8x</td>
<td>6.5</td>
<td>6.5</td>
<td>100.1</td>
</tr>
<tr>
<td>CSF</td>
<td>-</td>
<td>78.6</td>
<td>-</td>
<td>-</td>
</tr>
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<td></td>
<td>2x</td>
<td>32.5</td>
<td>39.3</td>
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<tr>
<td></td>
<td>8x</td>
<td>8.9</td>
<td>9.8</td>
<td>90.5</td>
</tr>
<tr>
<td>amniotic fluid</td>
<td>-</td>
<td>3605</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>1783</td>
<td>1802</td>
<td>98.9</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>906</td>
<td>901</td>
<td>100.6</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>444</td>
<td>451</td>
<td>98.6</td>
</tr>
</tbody>
</table>
14. DEFINITION OF THE STANDARD

Standard is calibrated against WHO 1st International Standard for Interleukin-6 89/548.

15. PRELIMINARY POPULATION AND CLINICAL DATA

Seventy serum samples from unselected healthy donors (35 men + 35 women) were assayed with the BioVendor Human Interleukin-6 ELISA in our laboratory. Sixty-seven samples were measured under the lowest standard 1.25 pg/ml and three samples were measured between 1.25 and 5 pg/ml.

- 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 IL-6 levels with the assay.

16. METHOD COMPARISON

The BioVendor Human Interleukin-6 ELISA was compared to another commercial immunoassay by measuring 49 serum 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
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>
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<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>🔄 8 °C</td>
<td>Storage conditions</td>
</tr>
<tr>
<td>🏛️</td>
<td>Name and registered office of the manufacturer</td>
</tr>
<tr>
<td>IVD</td>
<td>In vitro diagnostic medical device</td>
</tr>
</tbody>
</table>
**Assay Procedure Summary**

1. **Antibody Coated Microtiter Plate**
2. Reconstitute QCs and Master Standard and prepare set of standards
3. Add standards, QCs and samples 100 μl
4. Incubate at RT for 1 hour / 300 rpm
5. Wash 3x
6. Add Biotin Labelled Antibody solution 100 μl
7. Incubate at RT for 1 hour / 300 rpm
8. Wash 3x
9. Add Streptavidin HRP Conjugate 100 μl
10. Incubate at RT for 30 min / 300 rpm
11. Wash 3x
12. Add Substrate Solution 100 μl
13. Incubate at RT for 10 min
14. Add Stop Solution 100 μl
15. Read absorbance and calculate results

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**Additional Instructions**

- Dilute samples
- Prepare Wash Solution
- Prepare Biotin Labeled Antibody solution

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**Assay Procedure Details**

1. Add Biotin Labelled Antibody solution 100 μl
2. Incubate at RT for 1 hour / 300 rpm
3. Add Streptavidin HRP Conjugate 100 μl
4. Incubate at RT for 30 min / 300 rpm
5. Add Substrate Solution 100 μl
6. Incubate at RT for 10 min
7. Add Stop Solution 100 μl
8. Read absorbance and calculate results