HUMAN INSULIN RECEPTOR ELISA

Product Data Sheet
Cat. No.: RD191041200R
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 RD191041200R Human Insulin Receptor ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human insulin receptor.

Features

- It is intended for research use only
- The total assay time is less than 4.5 hours
- The kit measures total insulin receptor in serum and plasma (EDTA, citrate, heparin)
- Assay format is 96 wells
- 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.
Insulin receptor (IR) is an \( \alpha_2\beta_2 \)-disulfide linked tetrameric tyrosin kinase receptor located in the plasma membrane of target cells [1]. This glycoprotein is composed of two extracellular \( \alpha \)-subunits (731 amino acids; 135 kDa) containing the insulin binding site and two transmembrane \( \beta \)-subunits (620 amino acids; 95kDa) that possess intrinsic tyrosine kinase activity in their intracellular domains and transduce the insulin signal into the cell interior [1,2]. 

The human insulin receptor is involved in glucose homeostasis, cell growth and differentiation [3]. Binding of insulin leads to a conformational change of the receptor, resulting in ATP binding, autophosphorylation, and subsequent phosphorylation of insulin receptor substrate proteins that are linked to the action of two main signalling pathways. The PI3-K/Akt pathway is involved in the glucose transport to the cell, induction of proliferation or inhibition of apoptosis, while the Ras/MAPK pathway is involved mainly in the control of cell growth and differentiation [4].

Two insulin receptor variants are produced in mammals by alternative splicing: IR-A lacking exon 11 and the full length IR-B. The IR-A and IR-B isoforms show different ligand binding affinity. IR-A is a high-affinity receptor not only for insulin but also for IGF-II, while IR-B may be considered a specific receptor for insulin [5]. Both insulin receptor isoforms are coexpressed in cells, and the relative abundance of IR-A and IR-B is regulated by development stage- and tissue-specific factors. IR-A is predominantly expressed in fetal and cancer cells, whereas IR-B is predominantly expressed in well-differentiated tissues including liver, adipose tissue and skeletal muscle [6, 7]. Dysregulation of insulin receptor splicing, i.e., increased IR-A expression in adult life, may play an underestimated role in cancer progression. Insulin receptor is overexpressed in several tumors, including breast, colon, lung, ovary, and thyroid carcinomas. Moreover, human lymphocyte-derived malignant cells, such as the IM-9 cells, are abundantly endowed with high-affinity insulin receptors [7].

Circulating forms of several classes of receptor molecules and their fragments have been identified in human plasma. The human insulin receptor was found to be secreted into the incubation medium by various cultured cell lines [1] and Schaefer et al. reported that transgenic mice expressing and secreting the soluble ectodomain of human insulin receptor into the plasma showed chronic hyperglycemia [8]. Another study has shown that injection of the purified His-tagged human insulin receptor \( \alpha \)-subunit into veins of mice increased in the concentration of blood glucose [2].

The soluble human insulin receptor ectodomain, which contains \( \alpha \)-subunit and a extracellular part of \( \beta \)-subunit, has been observed in human plasma of healthy individuals and observed at significantly elevated levels in plasma of patients with elevated blood glucose [9]. Furthermore, the urinary soluble insulin receptor levels in patients with diabetes were also significantly higher than those in healthy volunteers and were significantly correlated with both urinary resistin and insulin levels [10].

Areas of investigation:
Diabetology
Oncology
4. TEST PRINCIPLE

In the BioVendor Human Insulin Receptor ELISA, standards and samples are incubated in a microtitrate plate wells pre-coated with polyclonal anti-human insulin receptor antibody. After 120 minutes incubation and a washing, biotin-labelled polyclonal anti-human insulin receptor antibody is added and incubated with captured insulin receptor for 60 minutes. After another washing, the 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 insulin receptor. A standard curve is constructed by plotting absorbance values against concentrations of insulin receptor standards, and concentrations of unknown samples are determined using this standard curve.

5. PRECAUTIONS

- For professional use only
- Wear gloves and laboratory coats when handling immunodiagnostic materials
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled
- This kit may contain components of human or animal origin. These materials should be handled as potentially infectious
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary
- The materials must not be pipetted by mouth
6. TECHNICAL HINTS

- Reagents with different lot numbers should not be mixed
- Use thoroughly clean glassware
- Use deionized (distilled) water, stored in clean containers
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent
- Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light
- Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements

7. REAGENT SUPPLIED

<table>
<thead>
<tr>
<th>Kit Components</th>
<th>State</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody Coated Microtiter Strips</td>
<td>ready to use</td>
<td>96 wells</td>
</tr>
<tr>
<td>Biotin Labelled Antibody</td>
<td>lyophilized</td>
<td>2 vials</td>
</tr>
<tr>
<td>Streptavidin-HRP Conjugate</td>
<td>ready to use</td>
<td>13 ml</td>
</tr>
<tr>
<td>Master Standard</td>
<td>lyophilized</td>
<td>2 vials</td>
</tr>
<tr>
<td>Dilution Buffer</td>
<td>ready to use</td>
<td>2 x 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)
- Precision pipettes to deliver 5 -1000 μl with disposable tips
- Multichannel pipette to deliver 100 μl with disposable tips
- Absorbent material (e.g. paper towels) for blotting the microtiter plate after washing
- Vortex mixer
- Orbital microplate shaker capable of approximately 300 rpm
- Microplate washer (optional). [Manual washing is possible but not preferable.]
- Microplate reader with 450 ± 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 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 Insulin Receptor Master Standard**

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of standard!!!

Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (do not foam).

The resulting concentration of insulin receptor in the stock solution is **30 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>30 ng/ml</td>
</tr>
<tr>
<td>250 µl of stock</td>
<td>250 µl</td>
<td>15 ng/ml</td>
</tr>
<tr>
<td>250 µl of 15 ng/ml</td>
<td>250 µl</td>
<td>7.5 ng/ml</td>
</tr>
<tr>
<td>250 µl of 7.5 ng/ml</td>
<td>250 µl</td>
<td>3.75 ng/ml</td>
</tr>
<tr>
<td>250 µl of 3.75 ng/ml</td>
<td>250 µl</td>
<td>1.88 ng/ml</td>
</tr>
<tr>
<td>250 µl of 1.88 ng/ml</td>
<td>250 µl</td>
<td>0.94 ng/ml</td>
</tr>
<tr>
<td>250 µl of 0.94 ng/ml</td>
<td>250 µl</td>
<td>0.47 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**

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of Biotin Labelled Antibody!!!

Reconstitute the lyophilized Biotin Labelled Antibody with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). Dilute Biotin Labelled Antibody Concentrate 100x with Dilution Buffer (e.g. 10 µl of Biotin Labelled Antibody Concentrate + 990 µl of Dilution Buffer for 8 wells).

**Stability and storage:**

Do not store the reconstituted and/or diluted Biotin Labelled Antibody solutions.

**Wash Solution Conc. (10x)**

Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution, e.g. 100 ml of Wash Solution Concentrate (10x) + 900 ml of distilled water for use of all 96-wells.

**Stability and storage:**

The diluted Wash Solution is stable 1 month when stored at 2-8°C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2-8°C.
10. **PREPARATION OF SAMPLES**

The kit measures human insulin receptor in serum and plasma (EDTA, citrate, heparin).

Samples can be assayed immediately after collection, or after long-term storage at -20°C or -70°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 samples.

**An appropriate dilution should be assessed by the researcher in advance to batch measurement. Recommended starting dilution is 5x.**

Dilute samples 5x with Dilution Buffer just prior to the assay, e.g. 30 µl of sample + 120 µl of Dilution Buffer for singlets, or preferably 50 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°C, or preferably at -70°C or lower 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 insulin receptor.

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

1. Pipet 100 µl of diluted Standards, Dilution Buffer (=Blank) and samples, preferably in duplicates, into the appropriate wells. See Figure 1 for example of work sheet.
2. Incubate the plate at room temperature (ca. 25°C) for 2 hours, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 5-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against a paper towel.
4. Pipet 100 µl of Biotin Labelled Antibody 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 5-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against a paper towel.
7. Pipet 100 µl of Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at room temperature (ca. 25°C) for 30 minutes, shaking at ca. 300 rpm on an orbital microplate shaker.
9. Wash the wells 5-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against a paper towel.
10. Add 100 µl of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
11. Incubate the plate for 20 minutes at room temperature. The incubation time may be extended [up to 30 minutes] if the reaction temperature is less than 20°C. Do not shake the plate during the incubation.
12. Stop the colour development by adding 100 µl of Stop Solution.
13. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 - 650 nm). Subtract readings at 630 nm (550 - 650 nm) from the readings at 450 nm. The absorbance should be read within 5 minutes following step 12.

Note 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 insulin receptor concentration of off-scale standards and samples. The readings at 405 nm should not replace the readings for samples that were “in range” at 450 nm.

Note 2: Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat four times. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.
<table>
<thead>
<tr>
<th></th>
<th>strip 1+2</th>
<th>strip 3+4</th>
<th>strip 5+6</th>
<th>strip 7+8</th>
<th>strip 9+10</th>
<th>strip 11+12</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Standard 30</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 15</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 7.5</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 3.75</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 1.88</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 0.94</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 0.47</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 insulin receptor (ng/ml) in samples.

Alternatively, the \textit{logit log} function can be used to linearize the standard curve (i.e. \textit{logit} of absorbance (Y) is plotted against \textit{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. 5 ng/ml (from standard curve) x 5 (dilution factor) = 25 ng/ml.

\textbf{Figure 2: Typical Standard Curve for Human Insulin Receptor ELISA.}
13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human Insulin Receptor 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 insulin receptor values in wells and is 0.173 ng/ml.
  *Dilution Buffer is pipetted into blank wells.

- **Limit of assay**
  Samples with absorbances exceeding the absorbance of the highest standard should be measured again with higher dilution. The final concentration of samples calculated from the standard curve must be multiplied by the respective dilution factor.

- **Specificity**
  The antibodies used in this ELISA are specific for human insulin receptor with no detectable crossreactivity to human insulin and IGF-I at 1000 ng/ml.

Presented results are multiplied by respective dilution factor.

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (ng/ml)</th>
<th>SD (ng/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.77</td>
<td>1.03</td>
<td>6.6</td>
</tr>
<tr>
<td>2</td>
<td>30.52</td>
<td>1.15</td>
<td>3.8</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (ng/ml)</th>
<th>SD (ng/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.39</td>
<td>0.86</td>
<td>5.6</td>
</tr>
<tr>
<td>2</td>
<td>25.90</td>
<td>0.63</td>
<td>2.4</td>
</tr>
</tbody>
</table>
### Spiking Recovery
Serum samples were spiked with different amounts of human insulin receptor and assayed.

<table>
<thead>
<tr>
<th>Sample</th>
<th><strong>Observed</strong> (ng/ml)</th>
<th><strong>Expected</strong> (ng/ml)</th>
<th><strong>Recovery O/E (%)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13.89</td>
<td>23.26</td>
<td>97.7</td>
</tr>
<tr>
<td></td>
<td>22.73</td>
<td>32.64</td>
<td>99.3</td>
</tr>
<tr>
<td></td>
<td>32.41</td>
<td>51.39</td>
<td>100.6</td>
</tr>
<tr>
<td>2</td>
<td>22.26</td>
<td>31.64</td>
<td>98.3</td>
</tr>
<tr>
<td></td>
<td>31.10</td>
<td>41.01</td>
<td>97.4</td>
</tr>
<tr>
<td></td>
<td>39.94</td>
<td>59.76</td>
<td>102.5</td>
</tr>
</tbody>
</table>

### Linearity
Serum samples were serially diluted with Dilution Buffer and assayed.

<table>
<thead>
<tr>
<th>Sample</th>
<th><strong>Dilution</strong></th>
<th><strong>Observed</strong> (ng/ml)</th>
<th><strong>Expected</strong> (ng/ml)</th>
<th><strong>Recovery O/E (%)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>28.81</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>13.95</td>
<td>14.40</td>
<td>96.8</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>7.46</td>
<td>7.20</td>
<td>103.5</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>3.71</td>
<td>3.60</td>
<td>102.9</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>34.99</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>17.43</td>
<td>17.50</td>
<td>99.6</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>8.77</td>
<td>8.75</td>
<td>100.3</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>4.54</td>
<td>4.37</td>
<td>103.8</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 (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>19.12</td>
<td>17.18</td>
<td>17.45</td>
<td>22.37</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>18.62</td>
<td>15.23</td>
<td>16.27</td>
<td>20.17</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>19.58</td>
<td>17.50</td>
<td>14.97</td>
<td>20.86</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>18.17</td>
<td>15.11</td>
<td>16.38</td>
<td>20.21</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>26.38</td>
<td>25.18</td>
<td>23.82</td>
<td>29.29</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>26.04</td>
<td>19.16</td>
<td>20.75</td>
<td>27.32</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>23.48</td>
<td>18.47</td>
<td>19.11</td>
<td>25.45</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>19.54</td>
<td>16.37</td>
<td>16.73</td>
<td>21.42</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>19.19</td>
<td>16.25</td>
<td>16.65</td>
<td>22.67</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>16.47</td>
<td>13.61</td>
<td>14.47</td>
<td>18.54</td>
<td></td>
</tr>
<tr>
<td>Mean (ng/ml)</td>
<td>20.66</td>
<td>17.41</td>
<td>17.66</td>
<td>22.83</td>
<td></td>
</tr>
<tr>
<td>Mean Plasma/Serum (%)</td>
<td>84.3%</td>
<td>85.5%</td>
<td>110.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coefficient of determination $R^2$</td>
<td>0.77</td>
<td>0.87</td>
<td>0.95</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 3: Insulin receptor levels measured using Human Insulin Receptor ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.
14. DEFINITION OF THE STANDARD

The Standard used in this kit is recombinant protein. The recombinant human insulin receptor, produced in HEK293, is 105.9 kDa protein consisting of 917 amino-acid residues of human insulin receptor (His28-Lys944 of HIR-A, whole subunit alpha and extracellular domain of subunit beta) and 10 additional amino acids.

15. PRELIMINARY POPULATION DATA

The following results were obtained when serum samples from 154 unselected donors (89 men + 65 women) 20–65 years old were assayed with the BioVendor Human Insulin Receptor ELISA in our laboratory.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (years)</th>
<th>n</th>
<th>Insulin Receptor (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>20-29</td>
<td>18</td>
<td></td>
<td>19.00</td>
<td>18.16</td>
<td>7.69</td>
<td>11.09</td>
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<td></td>
<td>30-39</td>
<td>26</td>
<td></td>
<td>15.92</td>
<td>15.02</td>
<td>4.51</td>
<td>10.88</td>
<td>33.41</td>
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<tr>
<td></td>
<td>40-49</td>
<td>31</td>
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<td>17.27</td>
<td>16.33</td>
<td>4.18</td>
<td>12.09</td>
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<tr>
<td></td>
<td>50-65</td>
<td>14</td>
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<td>15.81</td>
<td>15.44</td>
<td>2.34</td>
<td>10.94</td>
<td>20.42</td>
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<tr>
<td>Women</td>
<td>20-29</td>
<td>12</td>
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<td>16.66</td>
<td>16.05</td>
<td>4.86</td>
<td>12.92</td>
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<td>16.20</td>
<td>15.32</td>
<td>3.68</td>
<td>11.62</td>
<td>23.40</td>
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</table>
Figure 4: Human insulin receptor 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 insulin receptor levels with the assay.

16. METHOD COMPARISON

The BioVendor Human Insulin Receptor ELISA has not been compared to any commercial immunoassay.
17. TROUBLESHOOTING AND FAQs

**Weak signal in all wells**
Possible explanations:
- Omission of a reagent or a step
- Improper preparation or storage of a reagent
- Assay performed before reagents were allowed to come to room temperature
- Manual washing
- Improper wavelength when reading absorbance

**High signal and background in all wells**
Possible explanations:
- Improper or inadequate washing
- Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
- Incubation temperature over 30°C

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


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

<table>
<thead>
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<td>🌎</td>
<td>Name and registered office of the manufacturer</td>
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Assay Procedure Summary

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

Add Standards and samples 100 µl

Dilute samples 5x

Prepare Wash Solution

Prepare Biotin Labelled Antibody solution

Add Standards and samples 100 µl

Incubate at RT for 2 hours/300 rpm

Wash 5x

Add Biotin Labelled Antibody solution 100 µl

Incubate at RT for 1 hour/300 rpm

Wash 5x

Add Streptavidin-HRP Conjugate 100 µl

Incubate at RT for 30 min/300 rpm

Wash 5x

Add Substrate Solution 100 µl

Incubate at RT for 20 min

Add Stop Solution 100 µl

Read absorbance and calculate results
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To find the office closest to you, visit www.biovendor.com/contact