Human Insulin Receptor ELISA

- High sensitivity (0.173 ng/ml)
- Excellent analytical characteristics
- Validated for human serum and plasma (EDTA, citrate, heparin) samples
- Preliminary population data
Insulin receptor (IR) is an α2β2-disulfide linked tetrameric tyrosin kinase receptor located in the plasma membrane of target cells [1]. This glycoprotein is composed of two extracellular α-subunits (731 amino acids; 135 kDa) containing the insulin binding site and two transmembrane β-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 on 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 α-subunit into veins of mice increased in the concentration of blood glucose [2].
The soluble human insulin receptor ectodomain, which contains α-subunit and a extracellular part of β-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].

**Intended use**

The RD191041200R Human Insulin Receptor ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human insulin receptor.

- The total assay time is less than 4.5 hours
- The kit measures insulin receptor in human 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

**Clinical application**

- Diabetology
- Oncology

**Test principle**

In the BioVendor Human Insulin Receptor ELISA, Standards and samples are incubated in microtitration wells pre-coated with polyclonal anti-human insulin receptor antibody. After 120 minutes incubation followed by washing, biotin labelled polyclonal anti-human insulin receptor antibody is added and incubated with the captured insulin receptor for 60 minutes. 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 insulin receptor. A standard curve is constructed by plotting absorbance values against insulin receptor concentrations of Standards and concentrations of unknown samples are determined using this standard curve.
### 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>Observed (ng/ml)</th>
<th>Expected (ng/ml)</th>
<th>Recovery O/E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13.89</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>22.73</td>
<td>23.26</td>
<td>97.7</td>
</tr>
<tr>
<td></td>
<td>32.41</td>
<td>32.64</td>
<td>99.3</td>
</tr>
<tr>
<td></td>
<td>51.67</td>
<td>51.39</td>
<td>100.6</td>
</tr>
<tr>
<td>2</td>
<td>22.26</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>31.10</td>
<td>31.64</td>
<td>98.3</td>
</tr>
<tr>
<td></td>
<td>39.94</td>
<td>41.01</td>
<td>97.4</td>
</tr>
<tr>
<td></td>
<td>61.24</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 Dilution</th>
<th>Observed (ng/ml)</th>
<th>Expected (ng/ml)</th>
<th>Recovery O/E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>28.81</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>34.99</td>
<td>-</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:

![Graph showing concentration of insulin receptor (ng/ml) for different sample matrices](image)

### Summary of protocol

- Reconstitute Master Standard and Biotin Labeled Antibody and prepare set of Standards
- Dilute samples (5x)
- Add 100 μl Standards and samples
- Incubate at RT for 2 hours/300 rpm
- Wash plate 5 times
- Add 100 μl Biotin Labelled Antibody
- Incubate at RT for 1 hour/300 rpm
- Wash plate 5 times
- Add 100 μl Streptavidin-HRP Conjugate
- Incubate at RT for 30 min/300 rpm
- Wash plate 5 times
- Add 100 μl Substrate Solution
- Incubate at RT for 20 min
- Add 100 μl stop solution
- Read absorbance and calculate results
Cross-reactivity

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.

Preliminary Population Data

The following results were obtained when serum samples from 154 unselected donors (89 men + 65 women) 21 - 65 years old were assayed with the BioVendor Human Insulin Receptor ELISA in our laboratory.

Age and Sex Dependent Distribution of Hu Insulin Receptor

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (years)</th>
<th>n</th>
<th>Mean Insulin Receptor (ng/ml)</th>
<th>Median Insulin Receptor (ng/ml)</th>
<th>SD Insulin Receptor (ng/ml)</th>
<th>Min. Insulin Receptor (ng/ml)</th>
<th>Max. Insulin Receptor (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>20-29</td>
<td>18</td>
<td>19.00</td>
<td>18.16</td>
<td>7.69</td>
<td>11.09</td>
<td>48.36</td>
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<tr>
<td></td>
<td>30-39</td>
<td>26</td>
<td>19.92</td>
<td>15.02</td>
<td>4.51</td>
<td>10.88</td>
<td>33.41</td>
</tr>
<tr>
<td></td>
<td>40-49</td>
<td>31</td>
<td>17.27</td>
<td>16.33</td>
<td>4.18</td>
<td>12.09</td>
<td>32.51</td>
</tr>
<tr>
<td></td>
<td>50-65</td>
<td>14</td>
<td>15.81</td>
<td>15.44</td>
<td>2.34</td>
<td>10.94</td>
<td>20.42</td>
</tr>
<tr>
<td>Female</td>
<td>20-29</td>
<td>12</td>
<td>16.66</td>
<td>16.05</td>
<td>4.86</td>
<td>12.92</td>
<td>22.33</td>
</tr>
<tr>
<td></td>
<td>30-39</td>
<td>25</td>
<td>17.94</td>
<td>17.73</td>
<td>5.37</td>
<td>10.28</td>
<td>36.98</td>
</tr>
<tr>
<td></td>
<td>40-49</td>
<td>20</td>
<td>15.96</td>
<td>15.16</td>
<td>4.19</td>
<td>9.88</td>
<td>31.16</td>
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<tr>
<td></td>
<td>50-61</td>
<td>8</td>
<td>16.20</td>
<td>15.32</td>
<td>3.68</td>
<td>11.62</td>
<td>23.40</td>
</tr>
</tbody>
</table>

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.
References


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