Human Adiponectin ELISA

- High sensitivity (26 ng/ml)
- Excellent analytical characteristics
- Validated for human serum and plasma (EDTA, citrate, heparin)

Energy metabolism and body weight regulation
Metabolic syndrome
Type 2 diabetes
Coronary artery disease
Atherosclerosis
Adiponectin, also referred to as Acrp30, AdipoQ and GBP-28, is a recently discovered 244 aminoacid protein, the product of the apM1 gene, which is physiologically active and specifically and highly expressed in adipose cells. The protein belongs to the soluble defence collagen superfamily; it has a collagen-like domain structurally homologous with collagen VIII and X and complement factor C1q-like globular domain. Adiponectin forms homotrimers, which are the building blocks for higher order complexes found circulating in serum. Together, these complexes make up approximately 0.01% of total serum protein. Adiponectin receptors AdipoR1 and AdipoR2 have been recently cloned; AdipoR1 is abundantly expressed in skeletal muscle, whereas AdipoR2 is predominantly expressed in the liver. Paradoxically, adipose tissue-expressed adiponectin levels are inversely related to the degree of adiposity. Adiponectin concentrations correlate negatively with glucose, insulin, triglyceride concentrations, liver fat content and body mass index and positively with high-density lipoprotein-cholesterol levels, hepatic insulin sensitivity and insulin-stimulated glucose disposal. Adiponectin has been shown to increase insulin sensitivity and decrease plasma glucose by increasing tissue fat oxidation.

Clinical studies have shown that low adiponectin levels are associated with insulin resistance and precede the onset of type 2 diabetes. Diabetic patients have low levels of adiponectin and even lower levels of adiponectin were observed in patients with poorly controlled type 2 diabetes and in diabetic patients with coronary heart disease. Hypoadiponectinemia is also closely associated with the metabolic syndrome and with the hypertriglyceridemic waist. Non-alcoholic fatty liver disease is described as part of the metabolic syndrome and levels of adiponectin have inverse association with liver enzymes and fatty liver disease. The key finding is that low adiponectin serum levels predict type 2 diabetes independent of other risk factors.

Adiponectin also inhibits the inflammatory processes of atherosclerosis suppressing the expression of adhesion and cytokine molecules in vascular endothelial cells and macrophages, respectively. This adipokine plays a role as a scaffold of newly formed collagen in myocardial remodelling after ischaemic injury and also stimulates angiogenesis by promoting cross-talk between AMP-activated protein kinase and Akt signalling in endothelial cells. Low serum adiponectin levels are found in patients with coronary artery disease.

Moreover, high circulating levels of adiponectin are associated with decreased risk of myocardial infarction, independent of other factors.

Altogether, monitoring of adiponectin levels and monitoring of processes that affect its production or its receptors are promising targets for prevention and treatment of obesity, insulin resistance, hyperlipidemia and atherosclerosis.
**Intended use**

The RD195023100 Human Adiponectin ELISA is a competitive enzyme immunoassay for the quantitative measurement of human adiponectin.

- European Union: for in vitro diagnostic use
- Rest of the world: for research use only!
- The total assay time is less than 3 hours
- The kit measures total adiponectin in serum and plasma (EDTA, citrate, heparin)
- Assay format is 96 wells
- Quality Controls are human serum based
- Standards are recombinant adiponectin based
- Components of the kit are provided ready to use or concentrated

**Clinical application**

- Energy metabolism and body weight regulation
- Metabolic syndrome
- Type 2 diabetes
- Coronary artery disease
- Atherosclerosis

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**Test principle**

In the BioVendor Human Adiponectin ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with recombinant human adiponectin together with polyclonal anti-human adiponectin antibody conjugated to horseradish peroxidase (HRP). After washing step, the HRP conjugate bound to the adiponectin immobilized on the wells 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 inversely proportional to the adiponectin concentration. A standard curve is constructed by plotting absorbance values against adiponectin of standards, and concentrations of unknown samples are determined using this standard curve.

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**HUMAN ADIPOnectin ELISA**

**CAT. NO.: RD195023100**

<table>
<thead>
<tr>
<th>Assay format</th>
<th>Competitive ELISA, Immobilized antigen, 96 wells/kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td>Serum, plasma</td>
</tr>
<tr>
<td>Standards</td>
<td>0.1 to 10 µg/ml</td>
</tr>
<tr>
<td>Limit of detection</td>
<td>26 ng/ml</td>
</tr>
</tbody>
</table>
**Precision**

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (µg/ml)</th>
<th>SD (µg/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.71</td>
<td>0.69</td>
<td>5.9</td>
</tr>
<tr>
<td>2</td>
<td>12.28</td>
<td>0.481</td>
<td>3.9</td>
</tr>
</tbody>
</table>

Inter-assay (Run-to-Run) (n=8)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (µg/ml)</th>
<th>SD (µg/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.23</td>
<td>0.52</td>
<td>6.3</td>
</tr>
<tr>
<td>2</td>
<td>19.86</td>
<td>1.39</td>
<td>7.0</td>
</tr>
</tbody>
</table>

**Spiking recovery**

Serum samples were spiked with different amounts of human adiponectin and assayed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Observed (µg/ml)</th>
<th>Expected (µg/ml)</th>
<th>Recovery O/E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>10.39</td>
<td>10.10</td>
<td>102.9</td>
</tr>
<tr>
<td></td>
<td>15.57</td>
<td>15.10</td>
<td>103.1</td>
</tr>
<tr>
<td></td>
<td>23.19</td>
<td>25.10</td>
<td>92.4</td>
</tr>
<tr>
<td>2</td>
<td>10.94</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>16.18</td>
<td>15.94</td>
<td>101.5</td>
</tr>
<tr>
<td></td>
<td>21.14</td>
<td>20.94</td>
<td>101.0</td>
</tr>
<tr>
<td></td>
<td>30.02</td>
<td>30.94</td>
<td>100.3</td>
</tr>
</tbody>
</table>

**Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Observed (µg/ml)</th>
<th>Expected (µg/ml)</th>
<th>Recovery O/E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>18.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>9.28</td>
<td>9.02</td>
<td>102.8</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>4.39</td>
<td>4.51</td>
<td>97.3</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>2.53</td>
<td>2.26</td>
<td>112.7</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>23.56</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>10.15</td>
<td>11.78</td>
<td>86.2</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>5.64</td>
<td>5.89</td>
<td>95.8</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>3.08</td>
<td>2.94</td>
<td>104.5</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 (µg/ml)</th>
<th>EDTA Plasma (µg/ml)</th>
<th>Citrate Plasma (µg/ml)</th>
<th>Heparin Plasma (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.37</td>
<td>6.01</td>
<td>5.52</td>
<td>6.23</td>
</tr>
<tr>
<td>2</td>
<td>5.52</td>
<td>6.71</td>
<td>4.97</td>
<td>6.19</td>
</tr>
<tr>
<td>3</td>
<td>4.57</td>
<td>3.84</td>
<td>3.63</td>
<td>3.67</td>
</tr>
<tr>
<td>4</td>
<td>6.57</td>
<td>7.87</td>
<td>6.98</td>
<td>9.05</td>
</tr>
<tr>
<td>5</td>
<td>12.89</td>
<td>11.54</td>
<td>11.88</td>
<td>11.83</td>
</tr>
<tr>
<td>6</td>
<td>13.72</td>
<td>15.42</td>
<td>13.20</td>
<td>16.32</td>
</tr>
<tr>
<td>7</td>
<td>5.82</td>
<td>4.88</td>
<td>3.95</td>
<td>4.81</td>
</tr>
<tr>
<td>8</td>
<td>15.29</td>
<td>14.74</td>
<td>15.66</td>
<td>16.97</td>
</tr>
<tr>
<td>9</td>
<td>11.43</td>
<td>10.03</td>
<td>9.95</td>
<td>10.44</td>
</tr>
<tr>
<td>10</td>
<td>5.93</td>
<td>5.71</td>
<td>6.05</td>
<td>5.39</td>
</tr>
</tbody>
</table>

| Mean (µg/ml) | 8.9          | 8.7          | 8.2          | 9.4          |
| Mean Plasma/Serum (%) | - | 97.4 | 91.8 | 105.6 |

Coefficient of determination $R^2$ | - | 0.92 | 0.96 | 0.91 |

*Figure: Adiponectin levels measured using Human Adiponectin ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.*
Summary of protocol

- Reconstitute QCs and Master Standard and prepare set of standards
- Dilute samples 30x
- Add Standards, QCs and samples 50 μl + Add Conjugate Solution 50 μl
- Prepare Wash Solution
- Incubate at RT for 2 hours/300rpm
- Wash plate 3 times
- Add Substrate Solution 200 μl
- Incubate at RT for 10-15 min.
- Add Stop Solution 50 μl
- Read absorbance and calculate results

Normal Values

The following results were obtained when serum samples from 335 healthy donors were assayed with BioVendor’s Human Adiponectin ELISA.

<table>
<thead>
<tr>
<th>Sex</th>
<th>BMI (kg/m²)</th>
<th>n</th>
<th>Mean Adiponectin (µg/ml)</th>
<th>SD Adiponectin (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>&lt; 25</td>
<td>41</td>
<td>10.9</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>25-30</td>
<td>52</td>
<td>8.8</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>&gt; 30</td>
<td>23</td>
<td>8.3</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>115</td>
<td>9.5</td>
<td>3.9</td>
</tr>
<tr>
<td>Women</td>
<td>&lt; 25</td>
<td>92</td>
<td>13.6</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>25-30</td>
<td>56</td>
<td>13.9</td>
<td>8.6</td>
</tr>
<tr>
<td></td>
<td>&gt; 30</td>
<td>57</td>
<td>11.4</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>220</td>
<td>13.2</td>
<td>6.1</td>
</tr>
</tbody>
</table>

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 adiponectin levels with the assay.
Method Comparison

The BioVendor Human Adiponectin ELISA was compared to the other commercial immunoassays, by measuring of 38 serum samples, in two different ELISA. The following correlation graphs were obtained:

**Figure: Method comparison.**

The BioVendor’s Human Adiponectin ELISA, High Sensitivity (a sandwich ELISA, RD191023100) was compared with the BioVendor’s Human Adiponectin ELISA (a competitive ELISA, RD195023100), by measuring of 33 serum samples. The following correlation graph was obtained:

**Correlation of two BioVendor Human Adiponectin ELISAs**

*Figure: Method comparison with the other ELISA of the same company.*
QUANTITATIVE DETERMINATION OF HUMAN ADIPONECTIN ELISA

Related products

- RSHAKMAN011R Adiponectin HMW Mouse/Rat ELISA
- RD191023100 Adiponectin Human ELISA, High Sensitivity (Sandwich)
- RD172112100 Adiponectin Globular Human E. coli
- RD172023100-C Adiponectin HMW-rich Human HEK293, Tag free
- RD172029100 Adiponectin Human E. coli
- RD172023100 Adiponectin Human HEK293
- RD172023100-F Adiponectin Human HEK293 Solution
- RD27203100 Adiponectin Mouse HEK293
- RD272091100 Adiponectin Mouse, Trimeric form HEK
- RD162023050 Adiponectin NATIVE, Human Serum
- RD572023100 Adiponectin Porcine HEK293
- RD184023100-01 Adiponectin (HEK) Human, Goat Polyclonal Antibody
- RD184023100 Adiponectin (HEK) Human, Rabbit Polyclonal Antibody
- RD184023100-01 Adiponectin (HEK) Human, Sheep Polyclonal Antibody
- RD581023100 Adiponectin (HEK) Porcine, Rabbit Polyclonal Antibody
- RD584023100 Adiponectin (HEK) Porcine, Sheep Polyclonal Antibody
- RD1820231005E4 Adiponectin Human, Mouse Monoclonal Antibody, Clone: 5E4
- RD1820231007C11 Adiponectin Human, Mouse Monoclonal Antibody, Clone: 7C11
- RD181023220 Adiponectin Human, Rabbit Polyclonal Antibody
- RD184023100 Adiponectin (HEK) Human, Goat Polyclonal Antibody
- RD181023100 Adiponectin (HEK) Human, Rabbit Polyclonal Antibody
- RD184023100-01 Adiponectin (HEK) Human, Sheep Polyclonal Antibody
- RD581023100 Adiponectin (HEK) Porcine, Rabbit Polyclonal Antibody
- RD584023100 Adiponectin (HEK) Porcine, Sheep Polyclonal Antibody
- RD1820231005E4 Adiponectin Human, Mouse Monoclonal Antibody, Clone: 5E4
- RD1820231007C11 Adiponectin Human, Mouse Monoclonal Antibody, Clone: 7C11
- RD181023220 Adiponectin Human, Rabbit Polyclonal Antibody

References to this product

2. Gumanova NG, Gavrilova NE, Chemnizkivich OI, Kots AV, Metelskaya VA. Ratios of Leptin to Insulin and Adiponectin to Endothelin Are Sex-Dependently Associated with Extent of Coronary Atherosclerosis. Biomarkers. 2016 Jun 14; :1-26
References


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