Human Osteoprotegerin ELISA

- High sensitivity (0.03 pmol/l)
- Excellent analytical characteristics
- Validated for human serum and plasma samples (EDTA, citrate, heparin)
Osteoprotegerin (OPG, osteoclastogenesis inhibitory factor, OCIF) is a product of the TNFRSF11B gene, located on chromosome 8q24. OPG belongs to the TNF (tumor necrosis factor receptor) superfamily, that plays a key role in bone remodeling. Human OPG is a secreted glycoprotein composed of 401 amino acid residues. OPG exists as a disulfide-linked homodimer (120 kDa) or as a monomer (60 kDa). Both of these forms are active but the dimer is more bioactive than the monomer. In contrast to most members of the TNF receptor superfamily, OPG probably exists only in a soluble form. Its ligands are RANKL and TRAIL. Human OPG shares 85% amino acid identity to mouse OPG and 86 % identity to rat OPG. In adult humans OPG mRNA is highly expressed in bones (osteoblasts), endothelial vessel cells, skin, liver, stomach, intestine, heart, brain and lung and is also present in atherosclerotic plaques.

OPG and RANKL are involved in bone resorption and bone formation. OPG and receptor RANK compete with each other for binding to the ligand RANKL. Binding of RANKL to RANK stimulates osteoclasts and their activity. When RANKL binds to OPG, osteoclastogenesis decreases. OPG prevents the formation of RANKL/RANK, inhibits formation of osteoclasts and suppress bone resorption.

At normal physiological conditions OPG and ligand RANKL are in balance and bone resorption and bone formation are linked. This balance can be disrupted by the lack of estrogens in menopausal women, by anti-inflammatory effect of cytokines and by changes in the level of glucocorticoids, thyroid hormones, parathyroid hormone or calcitriol. Any modification in the RANKL/OPG ratio can induce either excessive bone resorption or, in contrast, excessive bone formation. This disregulation can lead to pathological conditions such as osteoporosis/osteopenia, bone tumor associated osteolysis, or cardiovascular pathology.

In postmenopausal osteoporosis, OPG serum level decreases and this decrease can be an indicator of a higher risk for bones fracture. In patients with glucocorticoid induced osteoporosis the RANKL/OPG ratio was higher. In patients with chronic obstructive pulmonary disease with low bone mineral density (BMD), RANKL/OPG ratio was significantly higher compared to those with normal BMD.

Patients with juvenile idiopathic arthritis had significantly lower levels of OPG in serum and lower OPG/RANKL ratio.

The OPG/RANKL/RANK system affects the cardiovascular system as well. In patients with ischemic heart disease the serum concentration of OPG was higher than that of healthy people. In patients with high OPG the risk of cardiovascular mortality is three- or four-times higher than it is in the healthy population.

Finally, the presence of malignant tumors leads to an inhibition of OPG production resulting in high bone resorption.

The OPG/RANKL/RANK system affects bone loss in many pathological states and participates in pathogenesis of vascular diseases. Determination of OPG concentration or RANKL/OPG ratio is a clinical indicator in the diagnosis of the pathological states mentioned below.
OSTEOPROTEGERIN ELISA

BioVendor Human Osteoprotegerin ELISA (RD194003200)

**Intended use**

The RD194003200 Human Osteoprotegerin ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human osteoprotegerin.

- European Union: for *in vitro* diagnostic use. Rest of the world: for research use only!
- The total assay time is less than 3.5 hours
- The kit measures osteoprotegerin in serum and plasma (EDTA, citrate, heparin)
- 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

**Test principle**

In the BioVendor Human Osteoprotegerin ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with monoclonal anti-human OPG antibody. After 60 minutes incubation and washing, biotin labelled polyclonal anti-human OPG antibody is added and incubated for 60 minutes with captured OPG. 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 OPG. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

**Clinical application**

- Bone and cartilage metabolism

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**HUMAN OSTEOPROTEGERIN ELISA**  
**CAT. NO.: RD194003200**

<table>
<thead>
<tr>
<th>Assay format</th>
<th>Sandwich ELISA, Biotin-labelled antibody, 96 wells/kit</th>
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<tbody>
<tr>
<td>Samples</td>
<td>Serum, plasma</td>
</tr>
<tr>
<td>Standards</td>
<td>1.5 to 60 pmol/l</td>
</tr>
<tr>
<td>Limit of detection</td>
<td>0.03 pmol/l</td>
</tr>
</tbody>
</table>

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![Absorbance at 450 nm](image-url)
**Precision**

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (pmol/l)</th>
<th>SD (pmol/l)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.26</td>
<td>0.41</td>
<td>2.9</td>
</tr>
<tr>
<td>2</td>
<td>4.82</td>
<td>0.18</td>
<td>3.8</td>
</tr>
<tr>
<td>3</td>
<td>12.72</td>
<td>0.31</td>
<td>2.5</td>
</tr>
<tr>
<td>4</td>
<td>15.28</td>
<td>0.74</td>
<td>4.9</td>
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</table>

Inter-assay (Run-to-Run) (n=3)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (pmol/l)</th>
<th>SD (pmol/l)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.83</td>
<td>0.34</td>
<td>7.1</td>
</tr>
<tr>
<td>2</td>
<td>6.18</td>
<td>0.55</td>
<td>9.0</td>
</tr>
<tr>
<td>3</td>
<td>12.93</td>
<td>0.69</td>
<td>5.3</td>
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<tr>
<td>4</td>
<td>14.33</td>
<td>0.25</td>
<td>1.7</td>
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**Spiking recovery**

Serum samples were spiked with different amounts of OPG and assayed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Observed (pmol/l)</th>
<th>Expected (pmol/l)</th>
<th>Recovery O/E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.38</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>8.12</td>
<td>6.89</td>
<td>117.9</td>
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<tr>
<td></td>
<td>14.73</td>
<td>13.92</td>
<td>105.8</td>
</tr>
<tr>
<td></td>
<td>20.48</td>
<td>20.71</td>
<td>98.9</td>
</tr>
<tr>
<td>2</td>
<td>11.38</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>13.57</td>
<td>12.89</td>
<td>105.3</td>
</tr>
<tr>
<td></td>
<td>20.93</td>
<td>19.92</td>
<td>105.1</td>
</tr>
<tr>
<td></td>
<td>28.62</td>
<td>26.71</td>
<td>107.2</td>
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</table>

**Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Observed (pmol/l)</th>
<th>Expected (pmol/l)</th>
<th>Recovery O/E (%)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>12.88</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>7.11</td>
<td>6.44</td>
<td>110.4</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>3.51</td>
<td>3.22</td>
<td>109.0</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>1.64</td>
<td>1.61</td>
<td>101.9</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>14.68</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>7.90</td>
<td>7.34</td>
<td>107.6</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>4.06</td>
<td>3.67</td>
<td>110.6</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>1.95</td>
<td>1.84</td>
<td>106.3</td>
</tr>
</tbody>
</table>

**Effect of sample matrix**

Citrate, heparin and EDTA plasmas were compared to respective serum samples from the same 10 individuals. Results are shown below:

**Summary of protocol**

- Reconstitute QCs and Master Standard and prepare set of Standards
- Dilute QCs and Standards 3x
- Dilute samples 3x
- Add Standards, QCs and samples 100 µl
- Prepare Wash Solution
- Incubate at RT for 1 hour / 300 rpm
- Wash 3x
- Add Biotin Labelled Antibody 100 µl
- Incubate at RT for 1 hour / 300 rpm
- Wash 3x
- Add Streptavidin-HRP Conjugate 100 µl
- Incubate at RT for 30 min / 300 rpm
- Wash 3x
- Add Substrate Solution 100 µl
- Incubate at RT for 10 min
- Add Stop Solution 100 µl
- Read absorbance and calculate results
Related products

- RBG10257010 Osteoprotegerin Human E. coli
- RD172003100 Osteoprotegerin Human HEK293
- RD182003110-01 Osteoprotegerin Human, Mouse Monoclonal Antibody, Clone: OPG-01
- RD182003110-13 Osteoprotegerin Human, Mouse Monoclonal Antibody, Clone: OPG-13
References


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