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OPG ELISA (Cat.No. BI-20403)

For the Determination of Osteoprotegerin in Human Samples

ASSAY CHARACTERISTICS

Method	Sandwich ELISA, HRP/TMB
Sample type	Serum, plasma (Citrate, EDTA, Heparin)
Standard range	0 – 20 pmol/l (0 /1.25 / 2.5 / 5 / 10 / 20 pmol/l)
Conversion factor	1 pg/ml = 0.05 pmol/l (MW: 19.9 kDa)
Sample volume	20 µl
Detection limit / LLOQ	0.07 pmol/l (0 pmol/l + 3 SD) / < 0.08pmol/l
Incubation time, temp.	4 h / 1 h / 30 min, room temperature
Cross reactivity	The assay does not cross react with rat or mouse samples.

Typical Standard Curve

Osteoprotegerin BI-20403
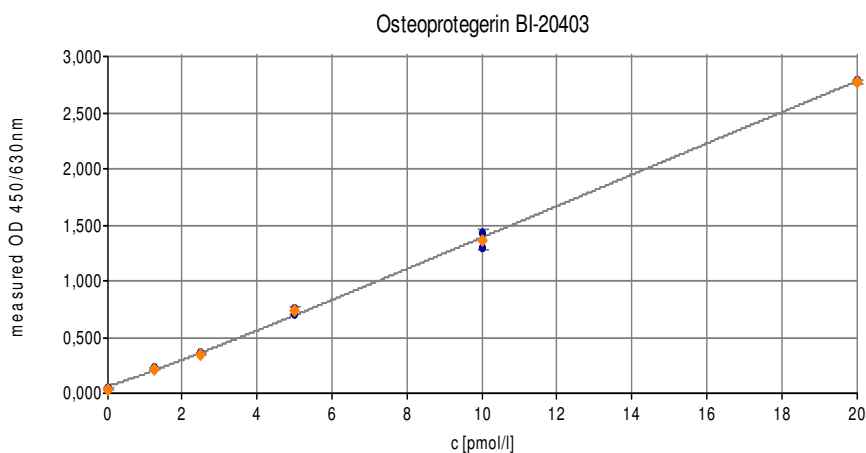
Values from apparently healthy individuals

Sample type	Serum (n=60)	EDTA plasma (n=6)	Heparin plasma (n=7)	Citrate plasma (n=5)
Median (pmol/l)	2.7	2.2	2.3	2.3

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PERFORMANCE CHARACTERISTICS

Spike Recovery

The mean recovery of recombinant OPG in serum and EDTA-plasma is between 93-108%.
The mean recovery of recombinant OPG in heparin- and citrate-plasma is between 82-109%.

Experiment: Recovery of spiked samples was tested by adding different concentrations of recombinant OPG (2, 5, 10 pmol/l) in 3 different serum and plasma human samples.

Data showing spike/recovery of serum samples:

matrix		serum				S/R (%)		
spike of recombinant OPG (pmol/l)		0	2	5	10	2	5	10
sample ID	#1	3.8	5.5	8.1	13.3	106%	94%	99%
	#2	2.6	4.5	8.2	12.3	95%	110%	97%
	#3	3.5	5.2	9.0	12.4	85%	111%	89%
Mean (%)						95%	105%	95%

Data showing spike/recovery of EDTA-plasma samples:

matrix		EDTA plasma				S/R (%)		
spike of recombinant OPG (pmol/l)		0	2	5	10	2	5	10
sample ID	#1	3.3	5.2	7.4	14.4	99%	83%	112%
	#2	2.5	4.6	6.7	14.4	102%	83%	119%
	#3	3.3	5.3	8.9	12.5	100%	113%	93%
Mean (%)						100%	93%	108%

Data showing spike/recovery of Lithium-Heparin plasma samples:

matrix		Lithium-Heparin plasma				S/R (%)		
spike of recombinant OPG (pmol/l)		0	2	5	10	2	5	10
sample ID	#1	3.1	4.4	6.6	11.5	67%	70%	84%
	#2	2.3	4.6	6.4	13.3	118%	82%	110%
	#3	3.1	4.9	7.9	12.0	87%	95%	89%
Mean (%)						91%	82%	94%

Data showing spike/recovery of citrate plasma samples:

matrix		Citrate plasma				S/R (%)		
spike of recombinant OPG (pmol/l)		0	2	5	10	2	5	10
sample ID	#1	2.7	4.4	7.7	12.6	85%	100%	99%
	#2	2.1	4.0	5.9	12.9	96%	76%	108%
	#3	2.8	4.4	7.,3	14.8	82%	90%	120%
Mean (%)						88%	89%	109%

Dilution Linearity:

The dilution linearity of endogenous OPG in serum samples (n=3) is between 79-102%.
The dilution linearity of recombinant OPG in serum samples (n=8) is between 80-116%.

Experiment: Dilution linearity was assessed by a serial dilution of serum samples with standard 1.

Data showing the dilution of endogenous OPG:

sample ID	reference	dilution 1+1		dilution 1+3		dilution 1+7	
	pmol/l	pmol/l	R (%)	pmol/l	R (%)	pmol/l	R (%)
#1	8.2	4.1	99%	1.9	94%	0.9	86%
#2	3.6	1.9	102%	0.8	92%	0.4	95%
#3	6.7	3.1	92%	1.4	86%	0.7	79%
Mean (%)			**98%**		**90%**		**87%**

Data showing the dilution of recombinant OPG:

sample ID	reference	spike of rec. OPG	dilution 1+1	
	pmol/l	6 pmol/l	pmol/l	R (%)
#1	3.7	10.7	5.2	98%
#2	2.3	6.7	3.9	116%
#3	4.4	12.8	5.1	80%
#4	3.4	12.0	5.1	86%
#5	3.3	9.4	5.0	107%
#6	3.6	10.3	5.2	101%
#7	4.4	11.6	4.9	84%
#8	4.7	10.9	5.2	96%
Mean (%)				**96%**

Intra-assay precision & Inter-assay precision

The intra-assay precision of the OPG ELISA is ≤ 3%.
The inter-assay precision of the OPG ELISA is ≤ 5%.

Experiment:
Intra-Assay: 2 samples with known concentrations were tested 5 times within 1 test system by
1 operator to assess intra-assay precision.

Experiment:
Inter-Assay: 2 samples with known concentrations were tested in total 12 times within 2 different kit
lots by 3 different operators to assess inter-assay precision.

Data showing Intra-Assay and Inter-Assay precision:

Intra-Assay (n=5)	Sample 1	Sample 2	Inter-Assay (n=12)	Sample 1	Sample 2
Mean (pmol/l)	3.2	10.1	Mean (pmol/l)	3.2	9.9
SD (pmol/l)	0.05	0.34	SD (pmol/l)	0.10	0.50
CV (%)	2%	3%	CV (%)	3%	5%

SAMPLE CHARACTERISTICS

Effect of sample matrix

Measurement of OPG in 4 different sample matrices from 7 samples of apparently healthy individuals showed a mean CV of 14%.
All 4 matrices can be tested by the assay.

Data showing the effect of the sample matrix:

Sample ID	pmol/l				Mean (pmol/l)	CV (%)
	serum	EDTA plasma	Heparin Plasma	Citrate plasma		
#1	2.1	2.4	3.2	n.a.	2.6	22%
#2	2.8	3.4	2.9	2.4	2.9	14%
#3	2.8	2.8	2.3	2.6	2.6	9%
#4	2.1	3.1	2.6	2.3	2.5	17%
#5	3.7	n.a.	4.2	3.4	3.8	11%
#6	0.9	1.0	0.8	0.8	0.9	11%
#7	0.9	0.7	0.7	n.a.	0.8	15%
n.a. = not available					**Mean CV**	**14%**

Stability of samples

We recommend performing serum or plasma separation by centrifugation as soon as possible (e.g. 20 min at 2000 x g, preferably at 4°C (2-8°C)). If this is not possible store the samples at 4°C (2-8°C) prior to centrifugation (up to one day).

The acquired serum or plasma samples should be measured as soon as possible. For longer storage aliquot samples and store at -25°C, for long time storage at -80°C. All samples should undergo only 4 freeze-thaw cycles. Serum samples can be stored for ≥ 2 years at -80°C.
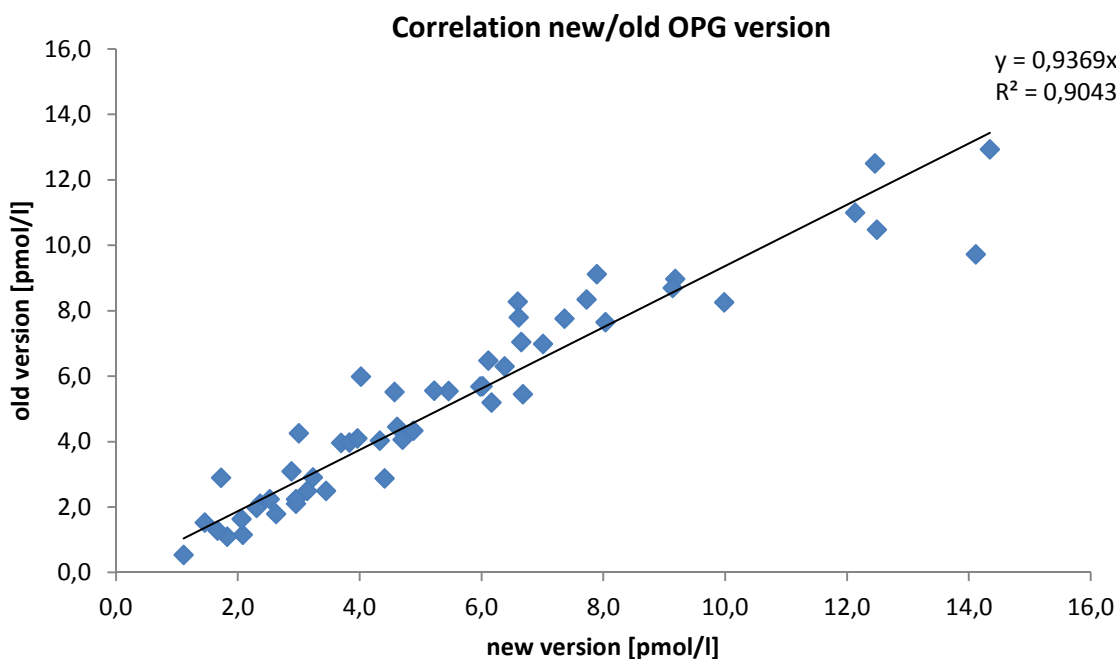
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Comparison Data:

OPG values in the old and new kit version

Samples measured in the old (BI-20402) and the new (BI-20403) OPG ELISA give comparable results.

Experiment: 52 serum samples were tested with the old (BI-20402) and the new (BI-20403) OPG ELISA.

Graph: comparison/correlation of the OPG concentrations of 52 samples tested by the old (BI-20402) and new (BI-20403) OPG ELISA.

Correlation new/old OPG version

$y = 0{,}9369x$
$R^2 = 0{,}9043$

old version [pmol/l] (y-axis), new version [pmol/l] (x-axis)

Number of serum samples (n=52)	OPG ELISA, old (cat. no. BI-20402)	OPG ELISA, new (cat. no. BI-20403)
	pmol/l	pmol/l
Mean	5.5	5.2
Median	4.7	4.8
Minimum	1.1	0.5
Maximum	14.3	12.9
90% Percentile	9.9	9.1

Date: March 2012