

PERIOSTIN ELISA

for the quantitative determination of Periostin
in human serum, EDTA plasma, heparin plasma, and citrate plasma
Cat. No. BI-20433 . 12 x 8 tests

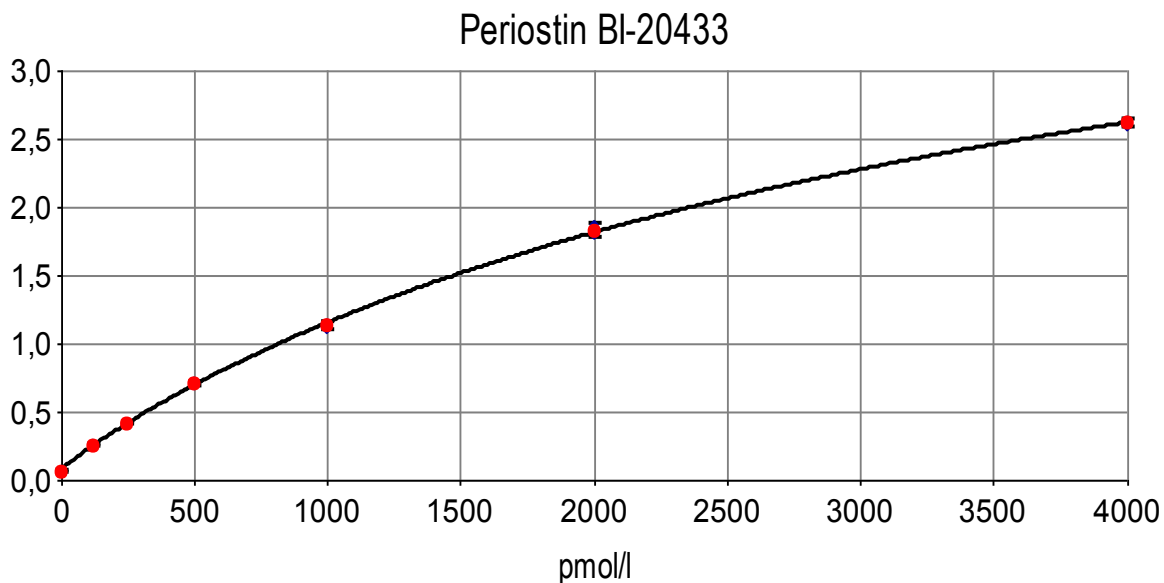
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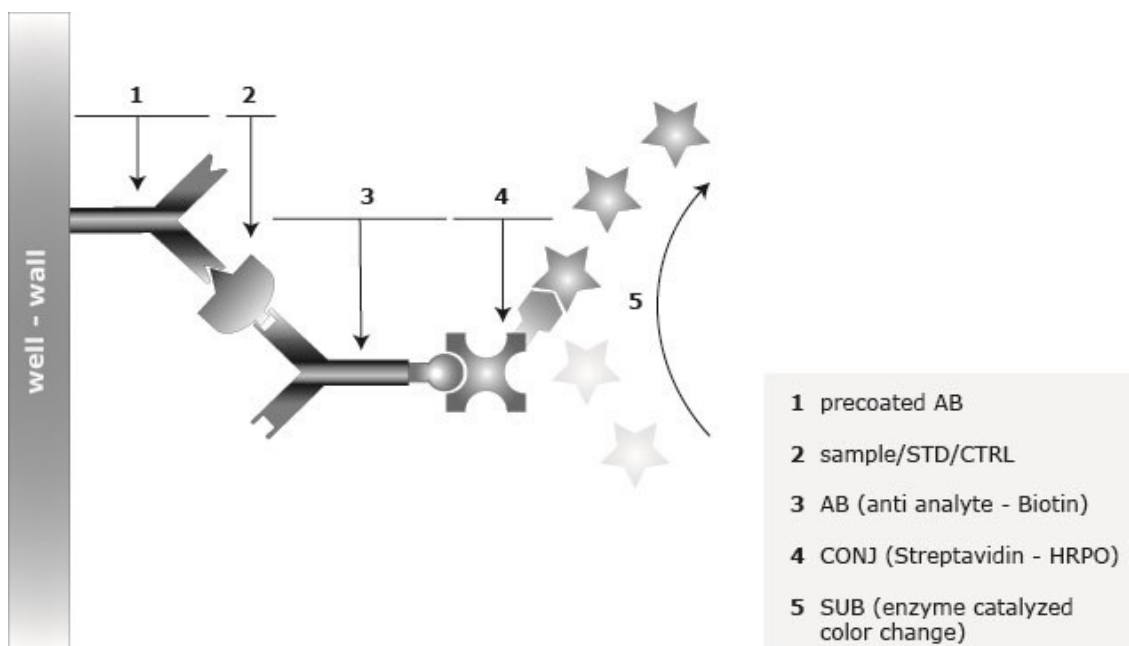
ASSAY CHARACTERISTICS Summary

Method	Sandwich ELISA, HRPO/TMB, 12x8-well strips		
Sample type	Serum, EDTA plasma, heparin plasma, and citrate plasma		
Standard range	0 to 4000 pmol/l (7 standards and 2 controls in a human serum matrix. Standards: (0, 125, 250, 500, 1000, 2000, 4000 pmol/l))		
Conversion factor	Periostin: 1 pg/ml = 0.011 pmol/l (MW: 91 kDa, endogenous)		
Sample volume	150 µl pre-diluted sample / well		
Incubation time, temp.	2 h / 2 h / 1 h / 30 min, room temperature		
Sensitivity	LOD (0 pmol/l + 3 SD): 20 pmol/l; LLOQ: 62.5 pmol/l		
Specificity	This assay is optimized to detect all known splicing forms of human Periostin. This assay recognizes recombinant and endogenous (natural) Periostin.		
Cross-reactivity	Due to the high sequence homology between human Periostin and Periostin of other species, the antibodies utilized in the assay may cross-react with mouse, rat, cynomolgous monkey, dog and cat Periostin.		
Calibration	This immunoassay is calibrated against recombinant human Periostin peptide.		
Precision	Intra-assay (n=5) ≤ 3% Inter-assay (n=10) ≤ 6%		
Average Spike/Recovery (spiked with two conc. of recombinant Periostin)	Sample type	Av. % Recovery (spike 500 pmol/l)	Av. % Recovery (spike 2000 pmol/l)
	Serum (n=7)	106	95
	EDTA plasma (n=8)	98	83
	Heparin plasma (n=7)	92	85
	Citrate plasma (n=8)	102	91
Dilution linearity of endogenous Periostin (samples pre-diluted 1+50 according to IFU)	Average % Recovery after dilution:		1+1 1+3
	Serum (n=12):		101 105
	EDTA plasma (n=4):		99 115
	Heparin plasma (n=4):		96 126
	Citrate plasma (n=4):		95 122
Values of apparently healthy individuals	Median serum (n=24) = 864 pmol/l Median EDTA plasma (n=20) = 817 pmol/l Median heparin plasma (n=20) = 891 pmol/l Median citrate plasma (n=24) = 885 pmol/l		

TYPICAL STANDARD CURVE



PRINCIPLE OF THE ASSAY



CAB coating antibody: monoclonal mouse IgG
DAB detection antibody: polyclonal goat IgG
AG antigen: Periostin protein

SAMPLE VALUES

Periostin levels in an apparently healthy cohort

	Periostin [pmol/l]			
	Serum (n=24)	EDTA plasma (n=20)	Heparin plasma (n=20)	Citrate plasma (n=24)
Mean	895	829	898	848
Median	864	817	891	885
Percentile 5%	422	533	573	359
Percentile 25%	711	689	729	643
Percentile 75%	1095	990	1064	975
Percentile 95%	1439	1105	1192	1357
Min	397	532	569	321
Max	1466	1109	1194	1407

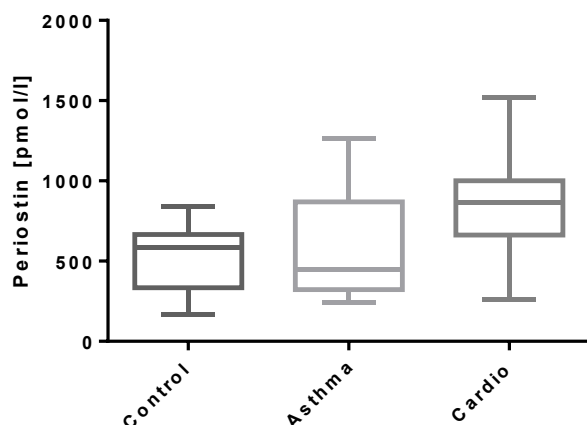
It is recommended to establish the normal range for each laboratory.

Periostin levels of various sample cohorts

Serum	Periostin [pmol/l]		
	Control (n=18)	Asthma Cohort* (n=10)	Cardio Cohort* (n=10)
Mean	508	572	849
Median	586	451	867
Percentile 25%	334	323	663
Percentile 75%	666	869	1002
Min	165	241	260
Max	840	1266	1521

*commercially available sample cohort (Seralab)

Sample Cohorts

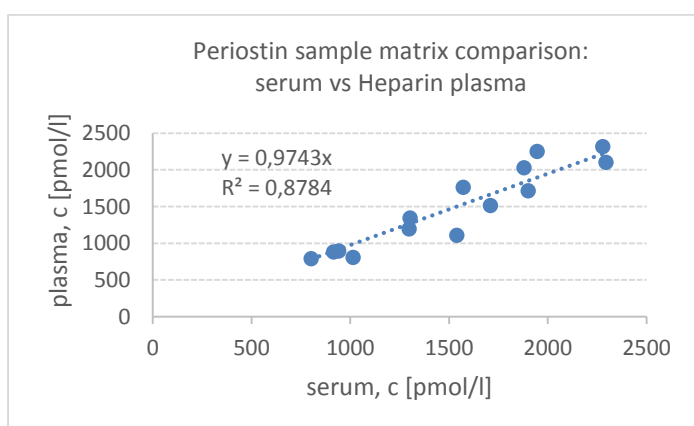
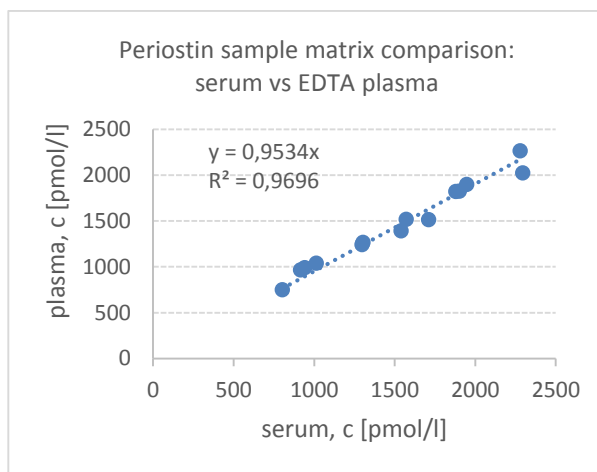
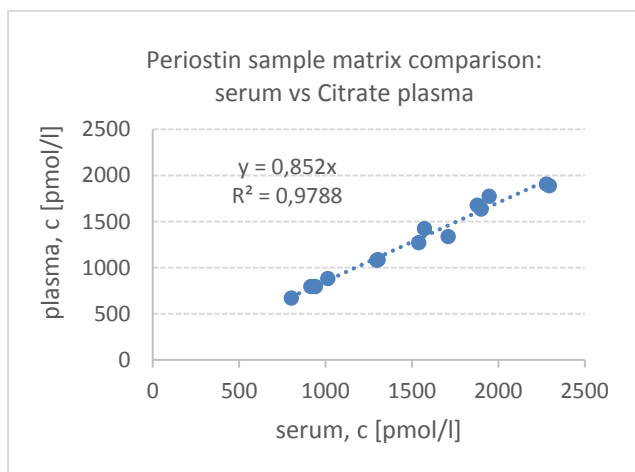


MATRIX COMPARISON

Correlation of serum and plasma samples from apparently healthy individuals

14 samples of apparently healthy individuals were prepared as serum and plasma pairs each deriving from one donor. Samples were assayed and the concentrations of the samples were compared.

Donor Sample ID	Periostin [pmol/]				CV [%]
	Serum	Citrate plasma	EDTA plasma	Heparin plasma	
#1	1297	1081	1243	1195	7
#2	1303	1087	1267	1345	8
#3	1710	1337	1514	1512	9
#4	1946	1775	1899	2251	9
#5	1539	1273	1392	1108	12
#6	2279	1906	2265	2316	8
#7	1901	1636	1825	1712	6
#8	1879	1678	1822	2028	7
#9	2295	1890	2026	2102	7
#10	1572	1425	1520	1760	8
#11	801	669	749	788	7
#12	1013	883	1039	806	10
#13	915	796	965	881	7
#14	941	794	992	894	8



ASSAY PERFORMANCE CHARACTERISTICS

RECOVERY

Summary of data showing mean recovery of human recombinant Periostin:

Sample matrix	Mean S/R [%]	
	+500 pmol/l	+2000 pmol/l
Serum (n=7)	106	95
EDTA plasma (n=8)	98	83
Heparin plasma (n=7)	92	85
Citrate plasma (n=8)	102	91

Experiments:

Recovery of spiked samples was tested by adding 2 known concentrations of human recombinant Periostin to different human sample matrices.

Data showing spike/recovery of human serum samples:

Sample ID	Spike Periostin [pmol/l]			S/R [%]	
	0	500	2000	500	2000
#S1	688	1044	2170	88	91
#S2	927	1251	2270	88	90
#S3	892	1417	2266	127	91
#S4	881	1257	2537	97	105
#S5	1138	1623	2500	126	97
#S6	1009	1471	2445	118	97
#S7	1107	1470	2437	100	94
Mean R [%]				106	95

Data showing spike/recovery of human EDTA plasma samples:

Sample ID	Spike Periostin [pmol/l]			S/R [%]	
	0	500	2000	500	2000
#E1	907	1272	2266	96	91
#E2	542	1083	1980	122	85
#E3	774	1272	2021	119	82
#E4	784	1304	2187	124	90
#E5	789	1045	2321	71	96
#E6	1145	1356	2116	71	77
#E7	968	1256	1917	82	72
#E8	1008	1390	1921	102	71
Mean R [%]				98	83

Data showing spike/recovery of human heparin plasma samples:

Sample ID	Spike Periostin [pmol/l]			S/R [%]	
	0	500	2000	500	2000
#H1	815	1201	2090	97	84
#H2	581	976	1878	94	79
#H3	765	1099	2090	86	85
#H4	808	1150	1908	89	75
#H5	831	1147	2345	84	96
#H6	780	1147	2067	93	84
#H7	998	1391	2304	104	90
Mean R [%]				92	85

Data showing spike/recovery of human citrate plasma samples:

Sample ID	Spike Periostin [pmol/l]			S/R [%]	
	0	500	2000	500	2000
#C1	798	1181	2036	97	82
#C2	519	956	1852	100	80
#C3	671	1074	2009	97	84
#C4	707	1136	1978	103	81
#C5	702	1113	2535	100	109
#C6	904	1332	2569	108	106
#C7	914	1209	2278	82	91
#C8	829	1369	2256	129	92
Mean R [%]				102	91

LINEARITY

Dilution linearity of samples containing endogenous human Periostin

Sample matrix	Mean R of dilution steps [%]	
	1+1	1+3
Serum (n=12)	101	105
EDTA plasma (n=4)	99	115
Heparin plasma (n=4)	96	126
Citrate plasma (n=4)	95	122

Recommendations for sample dilution

- We recommend diluting high measuring samples (outside of the calibration range) in assay buffer (ASYBUF, provided in the kit).

Experiment:

Dilution linearity was assessed by serially diluting samples containing endogenous human Periostin with assay buffer.

Data showing the dilution of endogenous Periostin in human serum samples:

Sample ID	Periostin [pmol/l]			R [%]	
	ref	1+1	1+3	1+1	1+3
#S1	647	308	121	95	93
#S2	1280	725	302	113	118
#S3	372	186	53	100	72
#S4	953	488	199	102	105
#S5	1440	713	316	99	110
#S6	1466	754	307	103	105
#S7	603	284	83	94	69
#S8	837	375	154	90	92
#S9	1608	846	511	105	127
#S10	1612	860	540	107	134
#S11	1155	618	337	107	117
#S12	1359	691	395	102	116
			Mean R [%]	101	105

Data showing the dilution of endogenous Periostin in human EDTA plasma samples:

Sample ID	Periostin [pmol/l]			R [%]	
	ref	1+1	1+3	1+1	1+3
#E1	1468	714	431	97	117
#E2	1328	669	377	101	114
#E3	1222	614	359	100	117
#E4	1677	826	460	98	110
			Mean R [%]	99	115

Data showing the dilution of endogenous Periostin in human heparin plasma samples:

Sample ID	Periostin [pmol/l]			R [%]	
	ref	1+1	1+3	1+1	1+3
#H1	1031	500	268	97	104
#H2	1230	597	544	97	177
#H3	1251	577	342	92	109
#H4	1417	682	407	96	115
			Mean R [%]	96	126

Data showing the dilution of endogenous Periostin in human citrate plasma samples:

Sample ID	Periostin [pmol/l]			R [%]	
	ref	1+1	1+3	1+1	1+3
#C1	999	461	309	92	124
#C2	993	504	305	101	123
#C3	1079	507	328	94	122
#C4	1163	534	340	92	117
			Mean R [%]	95	122

Dilution linearity of samples containing recombinant human Periostin:

Sample matrix pre-dilution acc. to assay protocol	Sample ID Dil. factor	Rec. Periostin 10,000 pmol/l			R [%]	
		Ref	1+4	1+9	1+4	1+9
Serum	#S1	1001	2342	1161	106	106
Serum	#S2	348	1765	956	85	92
Citrate plasma	#C1	665	1774	776	83	73
Citrate plasma	#C2	710	1918	858	90	80

Recommendations for sample dilution

- High measuring samples outside of the calibration range of the curve should be diluted in assay buffer (ASYBUF, provided in the kit).

PRECISION

Intra-assay precision & Inter-assay precision

Intra-assay (n=5) ≤ 3%, Inter-assay (n=10) ≤ 6%

Intra-assay: 2 samples of known concentrations were tested 5 times.

Inter-assay: 2 samples of known concentrations were tested 10 times within 3 kit lots by 3 different operators.

Intra-assay (n=5)	Sample 1	Sample 2	Inter-assay (n=10)	Sample 1	Sample 2
Mean (pmol/l)	249	2008	Mean (pmol/l)	251	1996
SD (pmol/l)	7.3	52	SD (pmol/l)	11.2	111.5
CV (%)	3	3	CV (%)	4	6

SENSITIVITY

Limit of detection (LOD)

The LOD is defined as the mean value of the back calculated concentration plus three times the standard deviation. The LOD of the Periostin ELISA is **20 pmol/l**.

Lower limit of quantification (LLOQ)

The LLOQ is defined as the accuracy of the back calculated concentrations and shall not exceed ±25% (acc. to ICH [Ref. 1]). The LLOQ of the Periostin ELISA is **62.5 pmol/l**.

SAMPLE STABILITY

Sample preparation

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).

The acquired serum or plasma samples should be measured as soon as possible. For longer storage aliquot samples and store at -25°C or lower. All samples should undergo only 4 freeze-thaw cycles.

Freeze/thaw of human samples containing endogenous Periostin:

No. of F/T cycles	0	2	3	4
Sample matrix	Mean Recovery [%]			
Serum (n=4)	100	100	103	98
EDTA plasma (n=4)	100	91	93	90
Citrate plasma (n=4)	100	93	91	97
Heparin plasma (n=4)	100	96	96	99

SPECIFICITY

This assay is optimized to detect all known splicing forms of human Periostin. This assay recognizes recombinant and endogenous (natural) Periostin.

CROSS-REACTIVITY

Due to the high sequence homology between human Periostin and Periostin of other species, the antibodies utilized in the assay may cross react with mouse, rat, cynomolgous monkey, dog and cat-Periostin.

CALIBRATION

This immunoassay is calibrated against recombinant human Periostin peptide.

Validation

The assay is fully validated according to ICH Q2 (R1), Ref. [1].

[1] CPMP/ICH/381/95 ICH Topic Q2 (R1) „Validation of Analytical Procedures: Text and Methodology” including:

ICH Q2A “Text on Validation of Analytical Procedures”

ICH Q2B “Validation of Analytical Procedures: Methodology”

Available on our Website www.bmgrp.com

Instructions for Use (package insert)

Material Safety Data Sheet

Periostin ELISA – Info Leaflet

Date: April 2016