

Periostin Mouse ELISA (Cat.No. BI-20433MS)

for the quantitative determination of mouse Periostin in serum and plasma samples Cat. No. BI-204033MS . 12×8 tests

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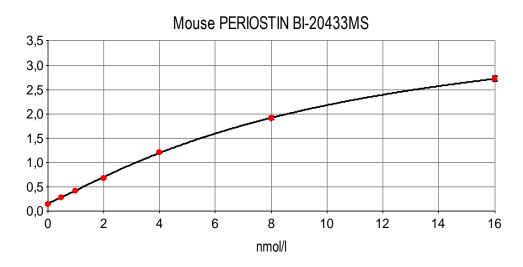
ASSAY CHARACTERISTICS SUMMARY

Method	Sandwich ELISA, F	HRPO/TMB, 12x	8-well strips			
Sample type	Mouse serum, plasma Protocol for cell culture samples on request.					
Standard range	0-16 nmol/l (0 / 0.	.5/1/2/4/8	3 / 16)			
Conversion factor	1 ng/ml = 0.011 n 1 nmol/l = 90.115	,				
Sample volume	≤ 5µl / sample					
Incubation time & temp.	3 h / 1 h / 30 min	- room temper	ature			
Sensitivity	LOD (0 nmol/l + 3 SD): 0.003 nmol/l; LLOQ: 0.125 nmol/l					
Specificity	This assay detects recombinant and endogenous mouse Periostin.					
Precision	Intra-assay (n=5) \leq 6%, Inter-assay (n=15) \leq 6%					
Spike/Recovery (average recovery spiked with 1,6 and 8	Mouse serum (n=4): 72%, 97%					
nmol/l recombinant mouse Periostin, respectively)	Mouse plasma (n=4): 86%, 88%					
Dilution linearity of	Recovery (%): Mouse Periostin rec. / endogenous			dogenous		
recombinant and endogenous mouse Periostin (average	Dilution:	1+1	1+3	1+7		
recovery of expected Periostin values after a 1+1; 1+3; 1+7	Mouse serum (n=4)	120 / 116	117 / 113	111 / *		
dilution with assay buffer)	Mouse plasma (n=4) 113 / 128 102 / 130 93 / *					
Periostin values from various mouse samples:	Adult mice (n=28): 3.4 ± 0.9 nmol/l 4-week old mice (n=24): 8.1 ± 1.0 nmol/l Female mice, 4-week old (n=13): 7.9 ± 1.2 nmol/l Male mice, 4-week old (n=11): 8.4 ± 0.7 nmol/l					

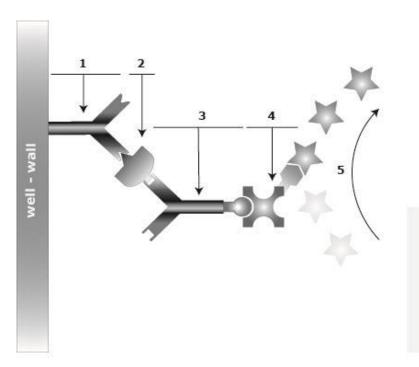
^{*}not detectable



TYPICAL STANDARD CURVE



PRINCIPLE OF THE ASSAY



- 1 precoated AB
- 2 sample/STD/CTRL
- 3 AB (anti analyte Biotin)
- 4 CONJ (Streptavidin HRPO)
- 5 SUB (enzyme catalyzed color change)

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



SAMPLE VALUES

PERIOSTIN VALUES OF "NORMAL" CONTROL MOUSE SERUM SAMPLES:

Samples Mouse serum	n	Periostin [nmol/l]
Adult mice	28	3.4 ± 0.9
4-week old mice	24	8.1 ± 1.0
Female, 4-week old mice	13	7.9 ± 1.2
Male, 4-week old mice	11	8.4 ± 0.7

Adult vs. 4-week old mice:

Mouse Periostin [nmol/l]	adult	4-weeks old
Number of serum samples	28	24
Minimum	1.6	5.3
Median	3.35	8.3
Maximum	5.4	10.1
5% Percentile	1.87	5.575
95% Percentile	5.31	10.08
Mean	3.468	8.171
Std. Deviation	0.9341	1.063

Female vs. male, panel: 4-week old mice

Mouse Periostin [nmol/I]	female	male
Number of serum samples	13	11
Minimum	5.3	7.6
Median	8	8.4
Maximum	10	10.1
Mean	7.908	8.491
Std. Deviation	1.245	0.7327

MATRIX COMPARISON SERUM VS. PLASMA LEVELS IN ADULT MOUSE SAMPLES:

Mouse Periostin [nmol/l]	serum	plasma
Number of serum samples	28	5
Minimum	1.6	1,3
Median	3.35	2.5
Maximum	5.4	2.9
Mean	3.468	2.4
Std. Deviation	0.9341	0.6403



ASSAY PERFORMANCE CHARACTERISTICS

RECOVERY

Recovery of mouse Periostin in mouse samples was evaluated. The spiked samples were diluted 1+200 as indicated in the package insert.

Summarv

Matrix n		Mouse Periostin (+1.6 nmol/l)		Mouse Periostin (+8 nmol/l)	
		Mean	Range	Mean	Range
Mouse serum	4	72%	60-86%	97%	88-107%
Mouse plasma	4	86%	67-96%	88%	70-100%

Spike recovery experiments in mouse samples

Recovery of spiked samples was tested by adding mouse recombinant Periostin to mouse serum samples. The spiked samples were diluted 1+200 as indicated in the package insert.

Data showing spike/recovery of mouse serum samples:

Sample ID	Spike mouse Periostin [nmol/l]			S/R [%]	
Sample ID	0	1.6	8	1.6	8
#MS1	1.7	2.7	7.8	79	88
#MS2	4.1	4.7	9.2	60	89
#MS3	3.4	4.0	10.2	64	107
#MS4	2.5	3.6	9.7	86	105
			Mean S/R [%]	72	97

Data showing spike/recovery of mouse plasma samples:

Sample ID	Spike	Spike mouse Periostin [nmol/l]			[%]
Sample ID	0	1.6	8	1.6	8
#MP1	2.9	3.6	7.0	67	70
#MP2	3.2	4.4	8.4	96	85
#MP3	2.6	3.7	9.1	85	98
#MP4	3.2	4.4	9.6	96	100
			Mean S/R [%]	86	88

DILUTION LINEARITY

Dilution linearity was assessed in mouse samples containing endogenous and recombinant mouse Periostin.

Summary of dilution linearity of samples spiked with recombinant mouse Periostin:

	R of dilution steps [%]				
matrix	1+1	1+3	1+7		
serum	120	117	111		
plasma	113	102	93		



Summary of dilution linearity of endogenous mouse Periostin:

	R of dilution steps [%]				
matrix	1+1	1+3			
serum	116	113			
plasma	128	130			

Experiments

Dilution linearity of the recombinant analyte:

Dilution linearity was assessed by diluting spiked mouse serum and plasma samples 1+1, 1+3, 1+7 with assay buffer. The sample preparation was performed according to the assay protocol.

Data showing the dilution of recombinant mouse Periostin in mouse serum samples

+8 nmol/l mouse P			eriostin R [%]				
Sample ID	ref	1+1	1+3	1+7	1+1	1+3	1+7
#MS1	10.1	6.8	3.3	1.5	135	130	118
#MS2	10.1	6.0	2.8	1.2	118	110	97
#MS3	11.5	6.6	33	1.6	115	114	112
#MS4	11.7	6.5	34	1.7	111	115	115
				Mean R [%]	120	117	111

Data showing the dilution of recombinant mouse Periostin in mouse plasma samples

Sample ID	+8 nmol/l mouse Periostin R				R [%]		
Sample ID	ref	1+1	1+3	1+7	1+1	1+3	1+7
#MP1	7.4	4.2	2.0	0.9	113	106	96
#MP2	8.2	4.5	2.1	0.9	110	99	89
#MP3	7.6	4.6	1.9	0.9	120	99	96
#MP4	8.1	4.4	2.1	0.9	109	106	91
	•			Mean R [%]	113	102	93

Dilution linearity of the endogenous analyte

Dilution linearity was assessed by diluting mouse serum and plasma samples 1+1, 1+3, with assay buffer. The sample preparation was performed according to the assay protocol.

Data showing the dilution of endogenous mouse Periostin in mouse serum

Sample ID	moi	use Perios	tin [nmol/l]	R [R [%]	
Sample 1D	ref	1+1	1+3	1+1	1+3	
#MS1	1.7	0.9	0.5	115	116	
#MS2	4.1	2.5	1.1	119	111	
#MS3	3.4	1.9	0.9	114	110	
#MS4	2.5	1.4	0.7	116	116	
			Mean R [%]	116	113	



Data showing the dilution of *endogenous* mouse Periostin in mouse plasma samples

Sample ID	mouse Periostin [nmol/l]			R [%]		
Sample ID	ref	1+1	1+3	1+1	1+3	
#MP1	2.9	2.1	1.2	147	163	
#MP2	3.2	1.9	0.9	121	108	
#MP3	2.6	1.6	0.7	122	114	
#MP4	3.2	2.0	1.1	123	134	
		_	Mean R [%]	128	130	

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

PRECISION

Intra-assay precision & Inter-assay precision

Intra-assay (n=5) \leq 6%, Inter-assay (n=15) \leq 6%

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

Inter-assay: 2 samples of known concentrations were tested 15 times within 2 kit lots by 2

different operators.

Intra-assay (n=5)	Sample 1	Sample 2
Mean (nmol/l)	1.0	7.8
SD (nmol/l)	0.06	0.14
CV (%)	6	2

Inter-assay (n=15)	Sample 1	Sample 2
Mean (nmol/l)	1.0	7.9
SD (nmol/l)	0.06	0.22
CV (%)	6	3

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 mouse ELISA is **0.003 nmol/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 mouse ELISA is **0.125 nmol/l**.

SPECIFICITY

This assay recognizes endogenous (natural) and recombinant mouse Periostin.

Experiments:

Samples containing endogenous Periostin were analysed.

The competition was performed by adding a >10-fold concentration of the coating antibody.



Competition of mouse serum and plasma samples containing endogenous mouse Periostin

	Mouse Perios	D. comp. [0/-1	
Sample ID	Reference	Competition	R comp. [%]
# MS1	9.2	0.00	100
# MS2	8.6	0.00	100
# MS3	10.1	0.00	100
# MS4	10.0	0.00	100
# MP1	2.9	0.00	100
# MP2	3.0	0.00	100
# MP3	2.8	0.00	100
# MP4	3.4	0.00	100
		Mean R [%]	100

Competition of a sample (Standard 7) containing recombinant mouse Periostin

	Mouse Perio	D.comp. [0/.1	
sample ID	Reference	Competition	R comp. [%]
Standard 7	12.6	0.1	99

CALIBRATION

This immunoassay is calibrated against purified recombinant mouse Periostin peptide.



SAMPLE CHARACTERISTICS

SAMPLE STABILITY

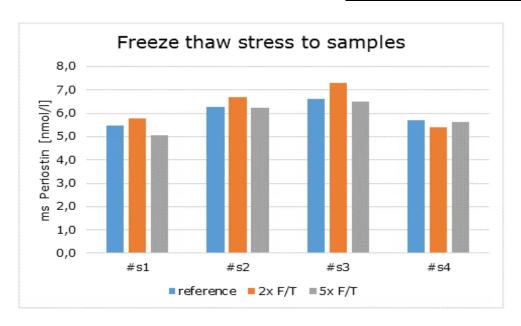
We recommend separating plasma or serum by centrifugation as soon as possible, e.g. 20 min at 2,000 x g, preferably at 4°C (2-8°C). Samples can be stored at 4°C (2-8°C) overnight. For long term storage, aliquot the acquired plasma or serum samples and store at -25°C or lower.

Samples can be subjected to 5 freeze-thaw cycles.

Aliquots of samples were stressed by freeze-thaw cycles. The reference is a sample which was not stressed. All samples were diluted according to protocol (1+200 diluted) shortly before testing.

Freeze-thaw stability of samples:

no of F/T cycles	Ref 0	2x	5x	
Sample ID	Mouse	Periostin	[nmol/l]	R 5x [%]
S1	5.5	5.8	5.0	92
S2	6.3	6.7	6.2	100
S3	6.6	7.3	6.5	98
S4	5.7	5.4	5.6	99
			Mean [%]	97

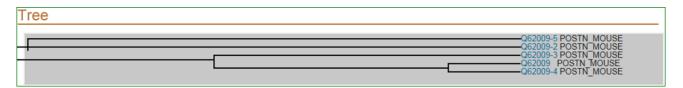


Undiluted serum samples are stable up to 5 freeze thaw cycles.



ISOFORMS OF MOUSE PERIOSTIN

Currently, 5 different isoforms of Mouse Periostin (http://www.uniprot.org/uniprot/Q62009) have been identified. They are generated by alternative splicing. (Recombinant mouse Periostin used in this assay shows the amino acid sequence of Q62009-1 (Isoform 1): 838AA, Mass (Da): 93,144.



SEQUENCE ALIGNMENT OF ISOFORMS 1,2,3,4,5:

Q62009 POSTN MOUSE	1	MVPLLPLYALLLLFLCDINPANANSYYDKVLAHSRIRGRDQGPNVCALQQILGTKKKYFS	60
Q62009-2 POSTN MOUSE	1	MVPLLPLYALLLFLCDINPANANSYYDKVLAHSRIRGRDOGPNVCALOOILGTKKKYFS	60
Q62009-3 POSTN MOUSE	ī	MVPLLPLYALLLLFLCDINPANANSYYDKVLAHSRIRGRDQGPNVCALQQILGTKKKYFS	60
Q62009-4 POSTN MOUSE	ī	MVPLLPLYALLLLFLCDINPANANSYYDKVLAHSRIRGRDQGPNVCALQQILGTKKKYFS	60
Q62009-5 POSTN_MOUSE	1	MVPLLPLYALLLLFLCDINPANANSYYDKVLAHSRIRGRDQGPNVCALQQILGTKKKYFS ************************************	60
Q62009 POSTN MOUSE	61	SCKNWYOGAICGKKTTVLYECCPGYMRMEGMKGCPAVMPIDHVYGTLGIVGATTTOHYSD	120
Q62009 POSIN MOUSE	61	SCKNWYQGAICGKKTTVLYECCPGYMRMEGMKGCPAVMPIDHVYGTLGIVGATTTQHYSD	120
Q62009-3 POSTN MOUSE	61	SCKNWYQGAICGKKTTVLYECCPGYMRMEGMKGCPAVMPIDHVYGTLGIVGATTTQHYSD	120
Q62009-4 POSTN_MOUSE	61	SCKNWYQGAICGKKTTVLYECCPGYMRMEGMKGCPAVMPIDHVYGTLGIVGATTTQHYSD	120
Q62009-5 POSTN_MOUSE	61	SCKNWYQGAICGKKTTVLYECCPGYMRMEGMKGCPAVMPIDHVYGTLGIVGATTTQHYSD ************************************	120
062009 POSTN MOUSE	121	VSKLREEIEGKGSYTYFAPSNEAWENLDSDIRRGLENNVNVELLNALHSHMVNKRMLTKD	180
Q62009-2 POSTN MOUSE	121	VSKLREEIEGKGSYTYFAPSNEAWENLDSDIRRGLENNVNVELLNALHSHMVNKRMLTKD	180
Q62009-3 POSTN MOUSE	121	VSKLREEIEGKGSTTTFAFSNEAWENLDSDIRRGLENNVNVELLNALHSHMVNKRMLTKD	180
Q62009-3 FOSIN MOUSE	121	VSKLREEIEGKGSTTTFAFSNEAWENLDSDIRRGLENNVNVELLNALHSHMVNKRMLTKD	180
Q62009-5 POSTN_MOUSE	121	VSKLREEIEGKGSYTYFAPSNEAWENLDSDIRRGLENNVNVELLNALHSHMVNKRMLTKD	180

Q62009 POSTN MOUSE	181	LKHGMVIPSMYNNLGLFINHYPNGVVTVNCARVIHGNQIATNGVVHVIDRVLTQIGTSIQ	240
Q62009-2 POSTN MOUSE	181	LKHGMVIPSMYNNLGLFINHYPNGVVTVNCARVIHGNQIATNGVVHVIDRVLTQIGTSIQ	240
Q62009-3 POSTN MOUSE	181	LKHGMVIPSMYNNLGLFINHYPNGVVTVNCARVIHGNOIATNGVVHVIDRVLTOIGTSIQ	240
Q62009-4 POSTN MOUSE	181	LKHGMVIPSMYNNLGLFINHYPNGVVIVNCARVIHGNQIATNGVVHVIDRVLTQIGISIQ	240
_	181		
Q62009-5 POSTN_MOUSE	181	LKHGMVIPSMYNNLGLFINHYPNGVVTVNCARVIHGNQIATNGVVHVIDRVLTQIGTSIQ	240
062009 POSTN MOUSE	241	DFLEAEDDLSSFRAAAITSDLLESLGRDGHFTLFAPTNEAFEKLPRGVLERIMGDKVASE	300
-			
Q62009-2 POSTN MOUSE	241	DFLEAEDDLSSFRAAAITSDLLESLGRDGHFTLFAPTNEAFEKLPRGVLERIMGDKVASE	300
Q62009-3 POSTN_MOUSE	241	DFLEAEDDLSSFRAAAITSDLLESLGRDGHFTLFAPTNEAFEKLPRGVLERIMGDKVASE	300
Q62009-4 POSTN_MOUSE	241	DFLEAEDDLSSFRAAAITSDLLESLGRDGHFTLFAPTNEAFEKLPRGVLERIMGDKVASE	300
Q62009-5 POSTN_MOUSE	241	DFLEAEDDLSSFRAAAITSDLLESLGRDGHFTLFAPTNEAFEKLPRGVLERIMGDKVASE ************************************	300
OCCORD DOGEN MONTH		ALLEGERAL OCCUPATION AND AN ARCHITECTURE AND ARCHITECTURE	0.50
Q62009 POSTN_MOUSE	301	ALMKYHILNTLQCSEAITGGAVFETMEGNTIEIGCEGDSISINGIKMVNKKDIVTKNGVI	360
Q62009-2 POSTN_MOUSE	301	ALMKYHILNTLQCSEAITGGAVFETMEGNTIEIGCEGDSISINGIKMVNKKDIVTKNGVI	360
Q62009-3 POSTN_MOUSE	301	ALMKYHILNTLQCSEAITGGAVFETMEGNTIEIGCEGDSISINGIKMVNKKDIVTKNGVI	360
Q62009-4 POSTN MOUSE	301	ALMKYHILNTLQCSEAITGGAVFETMEGNTIEIGCEGDSISINGIKMVNKKDIVTKNGVI	360
Q62009-5 POSTN_MOUSE	301	ALMKYHILNTLQCSEAITGGAVFETMEGNTIEIGCEGDSISINGIKMVNKKDIVTKNGVI ************************************	360
OCCORD DOCTH MOUGH	261	III TDEIII TDDGA VALITELA GVAAMMENDI HA OT GLA GGI VDDGEVIII LA DIBBIA EGDDET	420
Q62009 POSTN_MOUSE	361	HLIDEVLIPDSAKQVIELAGKQQTTFTDLVAQLGLASSLKPDGEYTLLAPVNNAFSDDTL	420
Q62009-2 POSTN_MOUSE	361	HLIDEVLIPDSAKQVIELAGKQQTTFTDLVAQLGLASSLKPDGEYTLLAPVNNAFSDDTL	420
Q62009-3 POSTN_MOUSE	361	HLIDEVLIPDSAKQVIELAGKQQTTFTDLVAQLGLASSLKPDGEYTLLAPVNNAFSDDTL	420
Q62009-4 POSTN_MOUSE	361	HLIDEVLIPDSAKQVIELAGKQQTTFTDLVAQLGLASSLKPDGEYTLLAPVNNAFSDDTL	420
Q62009-5 POSTN_MOUSE	361	HLIDEVLIPDSAKQVIELAGKQQTTFTDLVAQLGLASSLKPDGEYTLLAPVNNAFSDDTL	420
062009 POSTN MOUSE	421	CMPODITUTITONUTTWINGOTON VNCOTTENTOCNOTONININDAN TOTONICONINCON	480
		SMDQRLLKLILQNHILKVKVGLSDLYNGQILETIGGKQLRVFVYRTAICIENSCMVRGSK	
Q62009-2 POSTN MOUSE	421	SMDQRLLKLILQNHILKVKVGLSDLYNGQILETIGGKQLRVFVYRTAICIENSCMVRGSK	480
Q62009-3 POSTN_MOUSE	421	SMDQRLLKLILQNHILKVKVGLSDLYNGQILETIGGKQLRVFVYRTAICIENSCMVRGSK	480
Q62009-4 POSTN_MOUSE	421	SMDQRLLKLILQNHILKVKVGLSDLYNGQILETIGGKQLRVFVYRTAICIENSCMVRGSK	480
Q62009-5 POSTN_MOUSE	421	SMDQRLLKLILQNHILKVKVGLSDLYNGQILETIGGKQLRVFVYRTAICIENSCMVRGSK ************************************	480
Q62009 POSTN MOUSE	481	OCDMC) THI FDF TTOD) FYCI HDVI DODVDFCTFT CTT FAART VRITT TORCHUMT FARMS	540
		QGRNGAIHIFREIIQPAEKSLHDKLRQDKRFSIFLSLLEAADLKDLLTQPGDWTLFAPTN	
Q62009-2 POSTN_MOUSE	481	QGRNGAIHIFREIIQPAEKSLHDKLRQDKRFSIFLSLLEAADLKDLLTQPGDWTLFAPTN	540
Q62009-3 POSTN MOUSE	481	QGRNGAIHIFREIIQPAEKSLHDKLRQDKRFSIFLSLLEAADLKDLLTQPGDWTLFAPTN	540
Q62009-4 POSTN MOUSE	481	QGRNGAIHIFREIIQPAEKSLHDKLRQDKRFSIFLSLLEAADLKDLLTQPGDWTLFAPTN	540
Q62009-5 POSTN_MOUSE	481	QGRNGAIHIFREIIQPAEKSLHDKLRQDKRFSIFLSLLEAADLKDLLTQPGDWTLFAPTN	540



Q62009 POSTN MOUSE Q62009-2 POSTN MOUSE Q62009-3 POSTN MOUSE Q62009-4 POSTN MOUSE Q62009-5 POSTN MOUSE	541 541 541 541 541	DAFKGMTSEERELLIGDKNALQNIILYHLTPGVYIGKGFEPGVTNILKTTQGSKIYLKGV DAFKGMTSEERELLIGDKNALQNIILYHLTPGVYIGKGFEPGVTNILKTTQGSKIYLKGV DAFKGMTSEERELLIGDKNALQNIILYHLTPGVYIGKGFEPGVTNILKTTQGSKIYLKGV DAFKGMTSEERELLIGDKNALQNIILYHLTPGVYIGKGFEPGVTNILKTTQGSKIYLKGV DAFKGMTSEERELLIGDKNALQNIILYHLTPGVYIGKGFEPGVTNILKTTQGSKIYLKGV	600 600 600 600
Q62009 POSTN MOUSE Q62009-2 POSTN MOUSE Q62009-3 POSTN MOUSE Q62009-4 POSTN MOUSE Q62009-5 POSTN MOUSE	601 601 601 601 601	NETLLVNELKSKESDIMTTNGVIHVVDKLLYPADIPVGNDQLLELLNKLIKYIQIKFVRG NETLLVNELKSKESDIMTTNGVIHVVDKLLYPADIPVGNDQLLELLNKLIKYIQIKFVRG NETLLVNELKSKESDIMTTNGVIHVVDKLLYPADIPVGNDQLLELLNKLIKYIQIKFVRG NETLLVNELKSKESDIMTTNGVIHVVDKLLYPADIPVGNDQLLELLNKLIKYIQIKFVRG NETLLVNELKSKESDIMTTNGVIHVVDKLLYPADIPVGNDQLLELLNKLIKYIQIKFVRG ************************************	660 660 660 660
Q62009 POSTN_MOUSE Q62009-2 POSTN_MOUSE Q62009-3 POSTN_MOUSE Q62009-4 POSTN_MOUSE Q62009-5 POSTN_MOUSE	661 661 661 661	STFKEIPMTVYTTKIITKVVEPKIKVIQGSLQPIIKTEGPAMTKIQIEGDPDFRLIKEGE STFKEIPMTVYR	720 693 720 720 693
Q62009 POSTN MOUSE Q62009-2 POSTN MOUSE Q62009-3 POSTN MOUSE Q62009-4 POSTN MOUSE Q62009-5 POSTN MOUSE	721 694 721 721 694	TVTEVIHGEPVIKKYTKIIDGVPVEITEKQTREERIITGPEIKYTRISTGGGETGETLQK TVTEVIHGEPVIKKYTKIIDGVPVEITEKQTREERIITGPEIKYTRISTGGGETGETLQK TVTEVIHGEPVIKKYTKIIDGVPVEITEKQTREERIITGPEIKYTRISTGGGETGETLQK TVTEVIHGEPVIKKYTKIIDGVPVEITEKQTREERIIT TVTEVIHGEPVIKKYTKIIDGVPVEITEKQTREERIITGPEIKYTRISTGGGETGETLQK ************************************	780 753 780 758 753
Q62009 POSTN MOUSE Q62009-2 POSTN MOUSE Q62009-3 POSTN MOUSE Q62009-4 POSTN MOUSE Q62009-5 POSTN MOUSE	781 754 781 759 754	FLQKEVSKVTKFIEGGDGHLFEDEEIKRLLQGDTPAKKIPANKRVQGPRRRSREGRSQ FLQKEVSKVTKFIEGGDGHLFEDEEIKRLLQGDTPAKKIPANKRVQGPRRRSREGRSQ FLQ	838 811 810 784 783

This Mouse Periostin ELISA assay detects:

- Isoform 1 (mouse Periostin recombinant Standard/Calibrator utilized in this assay) Isoform 2 (due to sequence homology)
- Isoform 3 (due to sequence homology)
- Isoform 5 (experimentally analysed with recombinant protein).

Isoform 4 shows a change of sequence in the respective epitope of the coating antibody. Thus, it is not certain if this assay detects Isoform 4.

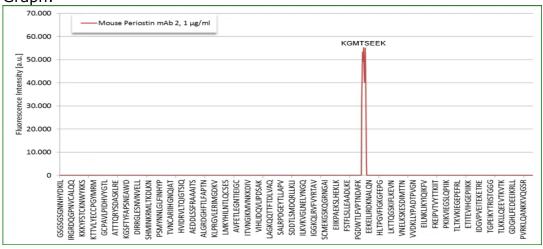
EPITOP MAPPING OF UTILIZED ANTIBODIES

Epitop mapping has been performed by Pepperprint.

Coating antibody:

The coating antibody which is a monoclonal mouse antibody binds to m_e1: KGMTSEER (AA544-551) of Q62009-1 (Isoform 1).







Detection antibody:

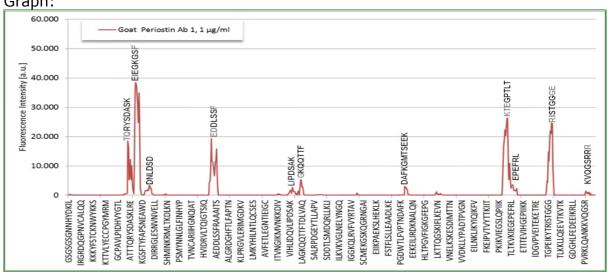
The detection antibody which is a polyclonal goat antibody binds to the following AA sequences of Q62009-1 (Isoform 1):

p_e1: TQHYSDVSK (AA115-123) p e2: EIEGKGSY (AA127-134) p_e3: EDDLSSF (AA246-252) p_e4: PAMT (AA672-676)

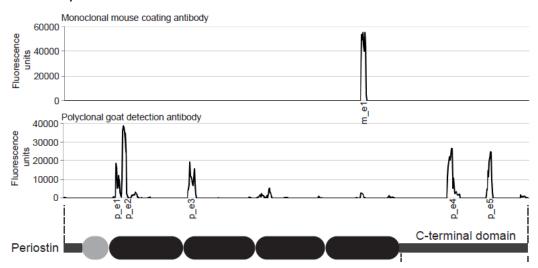
p_e5: RISTGGGE (AA738-746)

DAB binds to p_e1, p_e2, p_e3, p_e4: polyclonal goat IgG, anti-human periostin Ab



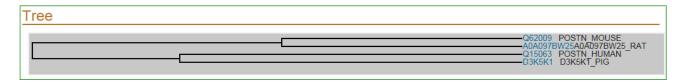


Summary:





INTERSPECIES ALIGNMENT



	Human	Mouse	Rat	Pig	
Human	100%				
Mouse	90%	100%			
Rat	90%	98%	100%		
Pig	95%	91%	92%	100%	

Human periostin isoform 1:	UniProt Q15063-1	7 isoforms described
Mouse periostin isoform 1:	UniProt Q62009-1	5 isoforms described
Rat periostin isoform 1:	UniProt A0A097BW25-1	1 isoform described
Pig periostin isoform 1:	UniProt D3K5K1	1 isoform described



Q15063 POSTN_HUMAN O62009 POSTN_MOUSE	1	MIPFLPMFSLLLLLIVNPINANNHYDKILAHSRIRGRDQGPNVCALQQILGTKKKYFS MVPLLPLYALLLLFLCDINPANANSYYDKVLAHSRIRGRDQGPNVCALQQILGTKKKYFS	58 60
D3K5K1 D3K5KT PIG	1	MIPFLPVFSLFLLVVVNPANANGHYDKILAHSRIRGRDQGPNVCALQQILGTKKKYFS	58
A0A097BW25 A0A097BW25_RAT	ī	MVPLLPLSALLLIFLCDVDPANANSYYDKVLAHSRIRGRDQGPNVCALQQILGTKKKYFS *:*:*::::::::::::::::::::::::::::::::	60
Q15063 POSTN HUMAN	59	TCKNWYKKSICGQKTTVLYECCPGYMRMEGMKGCPAVLPIDHVYGTLGIVGATTTQRYSD	118
Q62009 POSTN_MOUSE	61	SCKNWYQGAICGKKTTVLYECCPGYMRMEGMKGCPAVMPIDHVYGTLGIVGATTTQHYSD	120
D3K5K1 D3K5K1 PIG	59	TCKNWYQGAICGKKTTVLYECCPGYMRMEGMKGCPAVLPIDHVYGTLGIVGATTTQRYSD	118
A0A097BW25 A0A097BW25_RAT	61	SCKNWYQGAICGKKTTVLYECCPGYMRMEGMKGCPAVMPIDHVYGTLGIVGATTTQHYSD :****: :**:***************************	120
Q15063 POSTN_HUMAN	119	ASKLREEIEGKGSFTYFAPSNEAWDNLDSDIRRGLESNVNVELLNALHSHMINKRMLTKD	178
Q62009 POSTN MOUSE D3K5K1 D3K5KT PIG	121 119	VSKLREEIEGKGSYTYFAPSNEAWENLDSDIRRGLENNVNVELLNALHSHMVNKRMLTKD VSKLREEIEGKGSFTYFAPSNEAWDNLDSDIRRGLESNVNVELLNALHSHMVNKRMLTKD	180 178
A0A097BW25 A0A097BW25_RAT	121	VSKLREEIEGGSYTYFAPSNEAWDNLDSDIRRGLENNVNVELLNALHSHMVNKRMLTKD	180
Q15063 POSTN_HUMAN	179	LKNGMIIPSMYNNLGLFINHYPNGVVTVNCARIIHGNQIATNGVVHVIDRVLTQIGTSIQ	238
Q62009 POSTN MOUSE	181	LKHGMVIPSMYNNLGLFINHYPNGVVTVNCARVIHGNQIATNGVVHVIDRVLTQIGTSIQ	240
D3K5K1 D3K5K1 PIG A0A097BW25 A0A097BW25 RAT	179 181	LKNGMIIPSMYNNLGLFINHYPNGVVTVNCARIIHGNQIATNGVVHVIDRVLTQIGTSIQ LKHGMVIPSMYNNLGLFINHYPNGVVTVNCARVIHGNQIATNGVVHVIDRVLTQIGTSIQ	238 240
AUAUS/DWZJ AUAUS/DWZJ_RAI	101	**************************************	210
Q15063 POSTN_HUMAN O62009 POSTN_MOUSE	239 241	DFIEAEDDLSSFRAAAITSDILEALGRDGHFTLFAPTNEAFEKLPRGVLERIMGDKVASE	298
Q62009 POSTN MOUSE D3K5K1 D3K5KT PIG	239	DFLEAEDDLSSFRAAAITSDLLESLGRDGHFTLFAPTNEAFEKLPRGVLERIMGDKVASE DFIEAEDDLSSFRAAAITSDILESLGRDGHFTLFAPTNEAFEKLPRGVLERIMGDKVASE	300 298
A0A097BW25 A0A097BW25_RAT	241	DFIEAEDELSSFRAAAITSDLLESLGRDGHFTLFAPTNEAFEKLPRGVLERIMGDKVASE **:***:******************************	300
Q15063 POSTN HUMAN	299	ALMKYHILNTLQCSESIMGGAVFETLEGNTIEIGCDGDSITVNGIKMVNKKDIVTNNGVI	358
Q62009 POSTN_MOUSE	301	ALMKYHILNTLQCSEAITGGAVFETMEGNTIEIGCEGDSISINGIKMVNKKDIVTKNGVI	360
D3K5K1 D3K5K1 PIG A0A097BW25 A0A097BW25 RAT	299 301	ALMKYHILNTLQCSEAITSGAVFETLEGNTIEIGCDGDSITVNGIKMVNKKDIVTNNGAI ALMKYHILNTLQCSEAITGGAVFETMEGNTIEIGCEGDSISINGIKMVNKKDIVTKNGVI	358 360
AUAUS/DWZJ AUAUS/DWZJ_RAI	301	*************** * ********************	300
Q15063 POSTN_HUMAN	359	$\verb HLIDQVLIPDSAKQVIELAGKQQTTFTDLVAQLGLASALRPDGEYTLLAPVNNAFSDDTL $	418
Q62009 POSTN MOUSE	361	HLIDEVLIPDSAKQVIELAGKQQTTFTDLVAQLGLASSLKPDGEYTLLAPVNNAFSDDTL	420
D3K5K1 D3K5K1 PIG A0A097BW25 A0A097BW25_RAT	359 361	HLIDQVLIPDSAKQVIELAGNQQTTFTDLVAQLGLASALRPDGEYTLLAPVNNAFSDDTL HLIDEVLIPDSAKQVIELAGKQQTTFTDLVAQLGLASSLKPDGEYTLLAPVNNAFSDDTL ***:*********************************	418 420
Q15063 POSTN_HUMAN	419	SMDQRLLKLILQNHILKVKVGLNELYNGQILETIGGKQLRVFVYRTAVCIENSCMEKGSK	478
Q62009 POSTN MOUSE D3K5K1 D3K5KT PIG	421	SMDQRLLKLILQNHILKVKVGLSDLYNGQILETIGGKQLRVFVYRTAICIENSCMVRGSK	480
D3K5K1 D3K5K1 PIG A0A097BW25 A0A097BW25_RAT	419 421	SMDQRLLKLILQNHILKVKVGLNELYNGQKLETIGGKQLRVFVYRTAVCIENSCMVRGSK SMDQRLLKLILQNHILKVKVGLSDLYNGQILETIGGKQLRVFVYRTAICIENSCMVRGSK ************************************	478 480
Q15063 POSTN_HUMAN	479	QGRNGAIHIFREIIKPAEKSLHEKLKQDKRFSTFLSLLEAADLKELLTQPGDWTLFVPTN	538
Q62009 POSTN MOUSE D3K5K1 D3K5K1 PIG	481 479	QGRNGAIHIFREIIQPAEKSLHDKLRQDKRFSIFLSLLEAADLKDLLTQPGDWTLFAPTN	540
D3K5K1 D3K5K1 PIG A0A097BW25 A0A097BW25_RAT	481	QGRNGAIHIFREIIKPAEKSLHEKLKQDKRFSIFLSLLEAADLKELLTQPGDWTLFVPTN QGRNGAIHIFREIIQPAEKSLHEKLRQDKRFSIFLSLLEAADLKDLLTQPGDWTLFAPTN ************************************	538 540
Q15063 POSTN HUMAN	539	DAFKGMTSEEKEILIRDKNALQNIILYHLTPGVFIGKGFEPGVTNILKTTQGSKIFLKEV	598
Q62009 POSTN MOUSE	541	DAFKGMTSEERELLIGDKNALQNIILYHLTPGVYIGKGFEPGVTNILKTTQGSKIYLKGV	600
D3K5K1 D3K5K1_PIG A0A097BW25_A0A097BW25_RAT	539 541	DAFKGMTNEEKEILIRDKNALQNIILYHLTPGVFIGKGFEPGVTNILKTTQGSKIYLKGV DAFKGMTNEEREILIGDKNALQNIILYHLTPGVYIGKGFEPGVTNILKTTQGSKIYVKGV ****** **: ** ************************	598 600
Q15063 POSTN_HUMAN 062009 POSTN_MOUSE	599	NDTLLVNELKSKESDIMTTNGVIHVVDKLLYPADTPVGNDQLLEILNKLIKYIQIKFVRG	658
Q62009 POSTN MOUSE D3K5K1 D3K5KT PIG	601 599	NETLLVNELKSKESDIMTTNGVIHVVDKLLYPADIPVGNDQLLELLNKLIKYIQIKFVRG NDTLLVNELKSKESDIMTTNGVIHVVDKLLYPADIPVGNDOLLEILNKLIKYIOIKFVRG	660 658
A0A097BW25 A0A097BW25_RAT	601	NETLLVNELKSKESDIMTTNGVIHVVDKLLYPADIPVGNDQLLELLNKLIKYIQIKFVRG	660
Q15063 POSTN_HUMAN	659	STFKEIPVTVYTTKIITKVVEPKIKVIEGSLQPIIKTEGPTLTKVKIEGEPEFRLIKEGE	718
Q62009 POSTN MOUSE	661	STFKEIPMTVYTTKIITKVVEPKIKVIQGSLQPIIKTEGPAMTKIQIEGDPDFRLIKEGE	720
D3K5K1 D3K5KT PIG A0A097BW25 A0A097BW25 RAT	659 661	STFKEIPITVYRPMITKVKIEGEPELRLVKEGE STFKEIPMTVYRPAMTKIHIEGEPDFRLIKEGE	691 693
	501	************	330
Q15063 POSTN_HUMAN	719 721	TITEVIHGEPIIKKYTKIIDGVPVEITEKETREERIITGPEIKYTRISTGGGETEETLKK TVTEVIHGEPVIKKYTKIIDGVPVEITEKOTREERIITGPEIKYTRISTGGGETGETLOK	778 780
O62009 POSTN MOUSE		TVTEVINGEPVIKKITKIIDGVPVEITEKETREERIITGPEIKITRISTSGGETEETLKK	751
Q62009 POSTN MOUSE D3K5K1 D3K5KT PIG	692		
		TVTEVIHGEPVIKKYTKIIDGVPVEITEKETREERIITGPEIKYTRISTGGGETEETLQK *:***********************************	753
D3K5K1 D3K5KT PIG A0A097BW25 A0A097BW25_RAT Q15063 POSTN HUMAN	692 694 779	TVTEVIHGEPVIKKYTKIIDGVPVEITEKETREERIITGPEIKYTRISTGGGETEETLQK *:***********************************	836
D3K5K1 D3K5KT PIG A0A097BW25 A0A097BW25_RAT Q15063 POSTN_HUMAN Q62009 POSTN_MOUSE	692 694 779 781	TVTEVIHGEPVIKKYTKIIDGVPVEITEKETREERIITGPEIKYTRISTGGGETEETLQK *:***********************************	836 838
D3K5K1 D3K5KT PIG A0A097BW25 A0A097BW25_RAT Q15063 POSTN HUMAN	692 694 779 781 752	TVTEVIHGEPVIKKYTKIIDGVPVEITEKETREERIITGPEIKYTRISTGGGETEETLQK *:***********************************	836



VALIDATION

The assay is fully validated according to ICH Q2 (R1), Ref. (1,2).

REFERENCES

[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"

[2] Food and Drug Administration
Guidance for Industry, Bioanalytical Method Validation, Draft Guidance,
September 2013

Version: January 2019