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Validated and in-depth characterized sandwich ELISA for the quantification of mouse periostin

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CONCLUSION

This novel mouse periostin ELISA provides a reliable and accurate tool for the quantification of mouse periostin in minute amounts of serum and plasma samples.

SUMMARY

- Linear epitopes: coating antibody: epitope in Fas1 4; detection antibody: epitopes in Fas1 1-4 and C-terminus
- Isoforms: detection of isoforms 1, 2, 3 and 5 (4 not known)
- Sample volume: 0.5µl/well
- Calibrator: mouse periostin isoform 1
- Calibration range: 0.5 - 16 nmol/L
- Quantification limit: 0.125 nmol/L
- Specificity: 100% in serum and plasma
- Accuracy: Means for serum and plasma: 72% - 97%
- Dilution linearity: Means for endogenous periostin in serum and plasma: 113% - 130%
- Precision: intra-mediate: mean 5%; inter-mediate: mean 11%
- Measurements: Quantification of mouse periostin in serum and plasma, not in urine

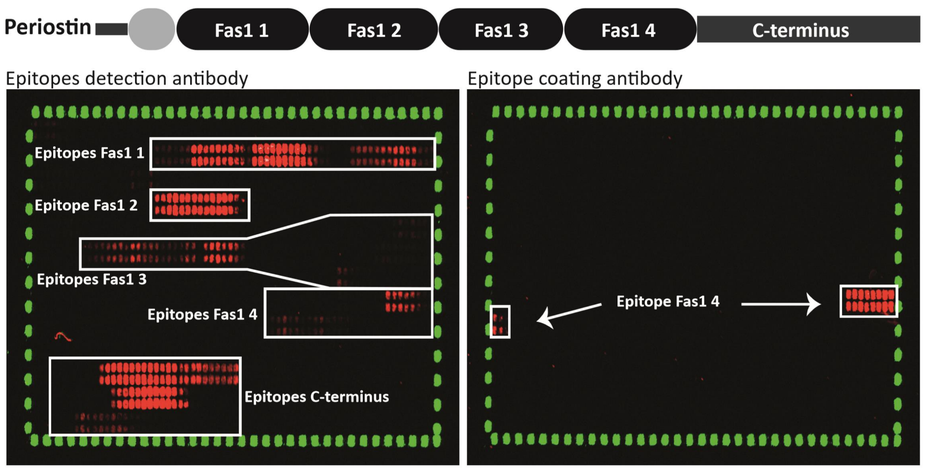
INTRODUCTION

Objective: Periostin (osteoblast-specific factor OSF-2) is an extracellular matrix protein which belongs to the FAS1 superfamily. It consists of a conserved N-terminus and a C-terminal region which is affected by alternative splicing leading to different isoforms. In bone, periostin is mainly expressed in the periosteum of long bones and in osteocytes. It has a general function in bone homeostasis and is regulated by factors like mechanical stimulation, PTH, growth factors and cytokines. It is upregulated during bone development and remodeling, and it acts on bone formation by increasing osteoblast function. To further study periostin in preclinical mouse models, there is the need for a high-quality assay for periostin quantification.

Methods: We developed a sandwich ELISA using polyclonal and monoclonal anti-periostin antibodies. Linear epitopes of the antibodies were resolved with microarray technology. Assay parameters like specificity, accuracy, dilution linearity, and precision were determined, and different sample matrices were tested (serum, plasma, urine). The binding pattern of periostin isoforms was analyzed.

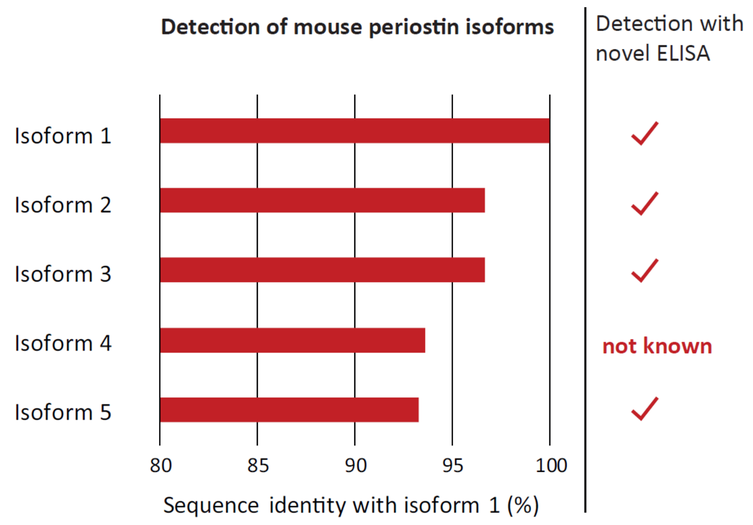
ANTIBODY CHARACTERISTICS

Mapping of linear epitopes revealed an epitope in Fas1 4 conserved between the isoforms for the monoclonal coating antibody, and multiple conserved epitopes for the polyclonal detection antibody (epitopes in the C-terminus may vary between the isoforms).



Top: Schematic representation of mouse periostin which is composed of an emilin (EMI) domain (light gray), four fasciclin-1 (Fas1) modules (black) and a C-terminal domain (dark gray) that is prone to alternative splicing.
Bottom: Linear epitopes of the detection and coating antibody were resolved with microarray technology. 15-mer peptides with an overlap of 14 amino acids covering the whole periostin isoform 1 sequence were printed horizontally in stacked duplicates on the microarray. Fluorescence signals of peptide-bound antibodies (red) and of control peptides flanking the array (green) are shown for the detection (left) and the coating antibody (right). Domain localization of the epitopes is indicated by framing.

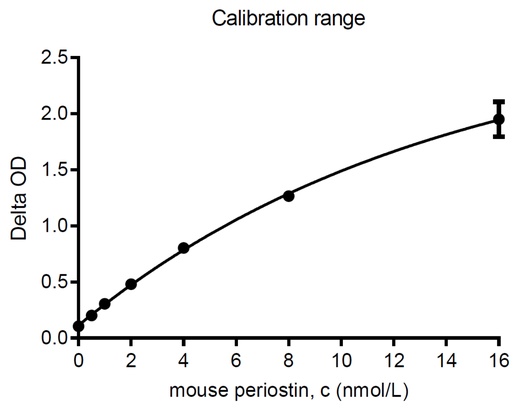
The sandwich ELISA is supposed to detect all currently known mouse periostin isoforms.



Five isoforms that are produced by alternative splicing are currently known for mouse periostin. Sequence alignment revealed sequence identities to isoform 1 of 96.7 % for isoform 2 and 3, and of 93.6 and 93.3, respectively, for isoform 4 and 5. The novel ELISA is able to detect isoform 1, 2, 3 and 5. Detection of isoform 4 is expected according to sequence analyses, but needs to be confirmed.

ASSAY PARAMETERS

The mouse periostin assay covers a range of 0.5 – 16 nmol/L.



Typical standard curve obtained with assay standards. The calibrator mouse periostin isoform 1 (x-axis, c = 0 – 16 nmol/L) was plotted versus OD values (y-axis).

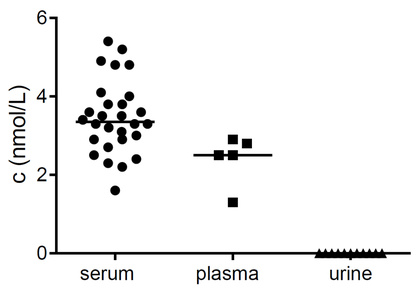
Determination of specificity, dilution linearity and accuracy shows that the assay is highly reliable and accurate.

Parameter	Definition	Serum (n)	Plasma (n)
Specificity	competition with coating antibody	100% (4)	100% (4)
Dilution linearity	1+1 dilution	116% (4)	128% (4)
	1+3 dilution	113% (4)	130% (4)
Accuracy	+1.6 nmol/L spike	72% (4)	86% (4)
	+8 nmol/L spike	97% (4)	88% (4)

Assay parameters were defined with samples from control mice. Specificity was tested by analyzing four serum and plasma samples in presence and absence of a surplus of coating antibody used for competition. Dilution linearity was assessed by diluting four serum and plasma samples with assay buffer. Accuracy (spike recovery) was tested by spiking 1.6 nmol/L and 8 nmol/L recombinant mouse periostin in four serum and plasma samples. Percent recoveries from concentrations were determined, means thereof are shown.

ASSAY APPLICATION

The sandwich ELISA assay detects periostin in mouse serum and plasma, but not in mouse urine.



28 serum, 5 plasma, and 11 urine samples (x-axis) from control mice were measured in the mouse periostin sandwich ELISA. Samples were analyzed with a sample pre-dilution of 1+200 in assay buffer. Concentrations (nmol/L) are shown on the y-axis. Results are displayed as scatter plot, lines within the chart represent median values.

LITERATURE

Takeshita et al. Osteoblast-specific factor 2: cloning of a putative bone adhesion protein with homology with the insect protein fasciclin I. Biochem J. (1993)

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