PERIOSTIN MOUSE

(EN) ELISA FOR THE QUANTITATIVE DETERMINATION OF MOUSE PERIOSTIN IN SERUM AND PLASMA
Cat. No. BI-20433MS . 12 x 8 TESTS

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

rev.no. 190117

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CONTENT

Detailed information on the Periostin mouse ELISA, e.g. assay validation data, sample matrix comparisons, and stability data is available on our website.

www.bmgrp.com

1) INTRODUCTION

Periostin (OSF-2) is secreted as a 91 kDa homodimeric soluble extracellular matrix protein expressed in collagen-rich fibrous connective tissues. Periostin is involved in osteoblast recruitment, attachment and spreading. It has been associated with the epithelial-mesenchymal transition in cancer and with the differentiation of mesenchyme in the developing heart. Periostin has functions in osteology, tissue repair, oncology, cardiovascular and respiratory diseases, and in various inflammatory settings. There are at least 7 isoforms of Periostin, caused by alternative splicing (http://www.uniprot.org/uniprot/Q15063).

Areas of interest:

- Bone diseases
- Tissue repair
- Cardiovascular diseases
- Respiratory diseases
- Cancer diseases

2) CONTENTS OF THE KIT

CONTENTS	KIT COMPONENTS	QUANTITY
PLATE	Mouse monoclonal anti-periostin antibody, pre-coated microtiter strips in a strip holder, packed in an aluminium bag with desiccant	12 x 8 tests
WASHBUF	Wash buffer concentrate 20x, natural cap	1 x 50 ml
ASYBUF	Assay buffer, red cap, ready to use	1x 110 ml
STOCK STD	Stock standard (16 nmol/l), recombinant mouse periostin, red cap, lyophilized	1 vial
CTRL	Control, yellow cap, lyophilized (exact concentration after reconstitution see label)	1 vial
AB	Goat polyclonal anti-periostin antibody, biotinylated, green cap, ready to use	1 x 7 ml
CONJ	Conjugate, (streptavidin-HRPO), amber bottle, amber cap, ready to use	1 x 18 ml
SUB	Substrate (TMB solution), amber bottle, blue cap, ready to use	1 x 22 ml
STOP	Stop solution, white cap, ready to use	1 x 7 ml

3) ADDITIONAL MATERIAL IN THE KIT

- 2 self-adhesive plastic films
- Quality control protocol
- Protocol sheet
- Instruction for use

4) MATERIAL AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

- Precision pipettes calibrated to deliver 5 µl, 50 µl, 100 µl, 200 µl, 300 µl and 1000 µl and disposable tips
- Distilled or deionised water
- Plate washer is recommended for washing, alternative multichannel pipette or manifold dispenser
- Refrigerator with 4°C (2-8°C)
- ELISA reader capable of measuring absorbance at 450 nm (with correction wavelength at 630 nm)
- Graph paper or software for calculation of results

5) REAGENTS AND SAMPLE PREPARATION

All reagents as supplied in the kit are stable at 4°C (2-8°C) until expiry date stated on the label of each reagent. Bring all reagents to room temperature before use.
Reconstituted STOCK STD and CTRL are stable at -25°C or lower until expiry date on label. STOCK STD and CTRL can undergo at least 3 freeze-thaw cycles.

Serum and plasma are suitable for use in this assay. Do not change sample type during studies.

A. Sample preparation:

Collect venous blood samples by using standardized blood collection tubes for serum or plasma. Perform serum and plasma separation by centrifugation according to supplier's instructions of the blood collection devices and measure the acquired serum or plasma samples as soon as possible. For longer storage aliquot and store at -25°C

or lower. Samples are stable for 5 freeze-thaw cycles. Thawed samples should be assayed as soon as possible. Lipemic or haemolysed samples may give erroneous results. Samples should be mixed well before assaying. We recommend duplicates for all values.

Samples must be diluted 1+200 with ASYBUF (assay buffer) prior to the assay, e.g. 5 µl sample + 1000 µl ASYBUF (or 2.5 µl sample + 500 µl ASYBUF). *Note: 100 µl pre-diluted sample is required per well.*

B. Preparation of CTRL (control):
Reconstitute the CTRL (control) in 200 µl distilled water, leave at room temperature (18-26°C) for 15 min and mix well prior to making dilution. The exact concentration is stated on the label.

CTRL must be diluted 1+200 with ASYBUF prior to the assay, e.g. 5 µl CTRL + 1000 µl ASYBUF (or 2.5 µl CTRL + 500 µl ASYBUF). *Note: 100 µl pre-diluted CTRL is required per well.*

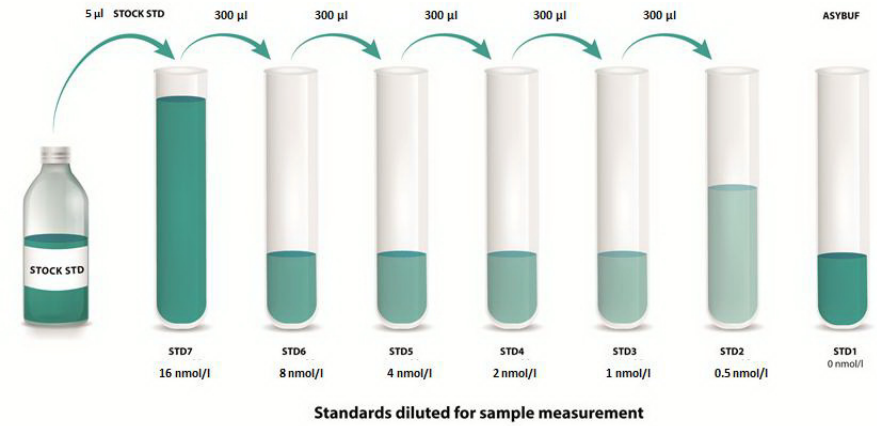
C. Preparation of STOCK STD (stock standard):
Reconstitute the mouse Periostin STOCK STD (stock standard) in 200 µl distilled water. Leave at room temperature (18-26°C) for 15 min and mix well prior to making dilutions.

D. Preparation of the standard curve:
Use polypropylene tubes
- Mark tubes as shown below (Graph1).
- Pipette 1000 µl of ASYBUF (assay buffer) into tube marked as STD7.
- Pipette 300 µl of ASYBUF each into tubes marked as STD6 to STD1

-.Pipette 5 µl of the reconstituted STOCK STD into tube marked as STD7. Mix thoroughly.
- Prepare a two-fold serial dilution to obtain STD6 to STD2. Pipette 300 µl STD7 into the tube labelled STD6. Mix thoroughly. Continue serial dilutions for STD5, STD4, STD3, STD2 (see Graph 1).
- ASYBUF serves as the zero standard (=STD1, 0 nmol/l).

Note: 100 µl pre-diluted STD is required per well.
Always mix each tube thoroughly before the next step!

Graph 1: Preparation of STD7 to STD1



Established standard curve already includes the 1+200 assay pre-dilution, thus the sample results can be read directly from the standard curve (also see chapter calculation of results).
Pre-diluted STDs, CTRL and samples should be measured as soon as possible (do not store or freeze).

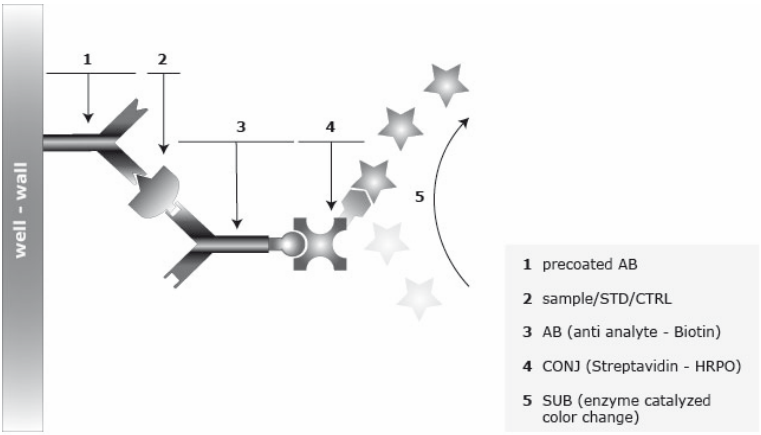
Preparation of WASHBUF (wash buffer):

Dilute the concentrate 1:20 (e.g. 50 ml WASHBUF + 950 ml distilled water). Crystals in the buffer concentrate will dissolve at room temperature (18-26°C). The undiluted WASHBUF is stable at 4°C (2-8°C) until expiry date stated on label. The diluted WASHBUF is stable up to one month at 4°C (2-8°C). Only use diluted WASHBUF when performing the assay.

For further information on sample stability please visit our website www.bmgrp.com (see Validation Data) or contact our customer service by e-mail info@bmgrp.com or by phone +43/ 1/ 29107-45.

6) PRINCIPLE OF THE ASSAY

This kit is a sandwich enzyme immunoassay for the quantitative determination of periostin in mouse serum and plasma samples. In a first step, STD/sample/CTRL and detection antibody (goat anti mouse Periostin-Biotin) are pipetted into the wells of the microtiter strips, which are pre-coated with monoclonal mouse anti Periostin antibody. Periostin present in the sample binds to the pre-coated antibody in the well and forms a sandwich with the detection antibody. In a wash step non-specific unbound material is removed. Then conjugate (Strepatvidin-HRPO) is added and reacts with the detection antibody. After another washing step, the substrate (TMB Tetramethylbenzidine) is pipetted into the wells. The enzyme catalysed colour change of the substrate is directly proportional to the amount of mouse Periostin present in the sample. This colour change is detectable with a standard microtiter plate ELISA reader. A dose response curve of the absorbance (optical density, OD at 450 nm) vs. standard concentration is generated, using the values obtained from the standard. The concentration of mouse Periostin in the sample is determined directly from the dose response curve.



7) ASSAY PROTOCOL

All reagents and samples must be at room temperature (18-26°C) before use in the assay.
Mark position for STD/SAMPLE/CTRL (Standard/Sample/Control) on the protocol sheet.
Take microtiter strips out of the aluminium bag. Store unused strips with desiccant at 4°C (2-8°C) in the aluminium bag. Strips are stable until expiry date stated on the label.

1. Pipette 100 µl of pre-diluted (1+200) STD/SAMPLE/CTRL (Standard/Sample/Control, see *5) reagents and sample preparation*) in duplicate into respective wells.
2. Add 50 µl AB (biotinylated anti-periostin antibody, green cap) into each well, swirl gently.
3. Cover tightly and incubate for 3 hours at room temperature (18-26°C).
4. Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer, natural cap). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel. .
5. Add 150 µl CONJ (Conjugate, amber cap) into each well, swirl gently.

6. Cover tightly and incubate for 1 hour at room temperature (18-26°C).
7. Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
8. Add 150 µl SUB (Substrate, blue cap) into each well, swirl gently
9. Incubate for 30 minutes at room temperature (18-26°C) in the dark.
10. Add 50 µl STOP (Stop solution, white cap) into each well, swirl gently.
11. Measure absorbance immediately at 450 nm (with reference 630 nm, if available).

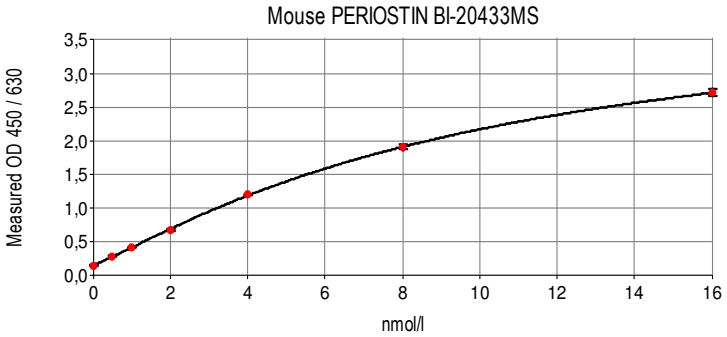
8) CALCULATION OF RESULTS

Read the optical density (OD) of all wells on a plate reader using 450 nm wavelength (correction wavelength 630 nm). Construct the standard curve from the OD values of the STD. Use commercially available software or graph paper. Obtain sample concentration from this standard curve. The assay was evaluated with logit-log and 4PL algorithm curve fitting. Different curve fitting methods need to be evaluated by the user.

Samples, control, and standards are all diluted 1+200 prior to the assay, so there is no need to take this dilution factor into account. Sample results can be read directly from the standard curve.

Note: Sample dilutions above 1+200 have to be considered when calculating the final sample concentration.

Example typical STD-curve:



The quality control (QC) protocol supplied with the kit shows the results of the final release QC for each kit lot. Data for OD obtained by customers may differ due to various influences and/or due to the normal decrease of signal intensity during shelf life. However, this does not affect validity of results as long as an OD of 1.00 or more is obtained for the STD with the highest concentration and the value of the CTRL is in range (target range see label).

9) ASSAY CHARACTERISTICS

Method:	Sandwich ELISA, HRP/TMB, 12x8-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 / 16)*
Conversion factor:	1 ng/ml = 0.011 nmol/l 1 nmol/l = 90.115 ng/ml
Sample volume:	≤ 5µl / sample
Incubation time and temperature:	3 h / 1 h / 30 min – room temperature
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) ≤ 6%, Inter-assay (n=15) ≤ 6%			
Spike/Recovery (average recovery spiked with 1.6 and 8 nmol/l rec. mouse Periostin, respectively):	Mouse serum (n=4): 72% , 97%			
	Mouse plasma (n=4): 86% , 88%			
Dilution linearity of recombinant and endogenous mouse Periostin (average recovery of expected Periostin values after a 1+1; 1+3; 1+7 dilution in assay buffer):	Recovery (%):	Mouse Periostin recombinant / endogenous		
	Dilution:	1+1	1+3	1+7
	Mouse serum (n=4)	120 / 116	117 / 113	111 / *
	Mouse plasma (n=4)	113 / 128	102 / 130	93 / *
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*

For further information on assay and sample characteristics please visit our website www.bmgrp.com (see Validation Data) or contact our customer service by e-mail info@bmgrp.com or by phone +43/ 1/ 29107-45.

10) PRECISION

Intra-assay: 2 samples of known concentration were tested 5 times in 1 kit lot by 1 operator.
Inter-assay: 2 samples of known concentration were tested 15 times in 3 different assays in 3 days 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 (npmol/l)	1.00	7.9
SD (nmol/l)	0.06	0.22
CV (%)	6	3

Detailed information on the mouse Periostin ELISA, e.g. assay performance characteristics, matrix comparisons, and stability data is available on our website www.bmgrp.com (see Validation Data).

11) TECHNICAL HINTS

- Do not mix or substitute reagents with those from other lots or sources.
- Do not mix stoppers and caps from different reagents or use reagents between lots.
- Do not use reagents beyond expiration date.
- Protect reagents from direct sunlight.
- Substrate solution should remain colourless until added to the plate.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.

12) PRECAUTIONS

All liquid reagents contain ≤ 0.1% Proclin 950 as preservative. Avoid contact with skin and mucous membrane. Proclin 950 is not toxic in concentrations used in this kit. It may cause allergic skin reactions – avoid contact with skin or eyes.

- Do not pipette by mouth.
- Do not eat, drink, smoke, or apply cosmetics where reagents are used.
- Wear gloves, glasses, and lab coat while performing this assay.
- Sulfuric acid is irritating to the eyes and skin. Avoid contact with skin and mucous. Irritations are possible. Flush with water if contact occurs.

13) LITERATUR

1. The role of periostin in tissue remodeling across health and disease. Conway SJ et al., Cell Mol Life Sci, 2014; 71(7): 1279-1288.
2. Serum periostin is associated with fracture risk in postmenopausal women: a 7-year prospective analysis of the OFELY study. Rousseau JC et al., J Clin Endocrinol Metab, 2014; 2533-2539.
3. Circulating periostin levels in patients with AS: association with clinical and radiographic variables, inflammatory markers and molecules involved in bone formation. Sakellariou GT et al., Rheumatology, 2015; 54: 908-914.
4. Serum Periostin: A Novel Biomarker for Asthma Management. Hisako Matsumoto, Allergology International, 2014; 63:153-160.
5. Serum periostin in obstructive airways disease. Fingleton J et al., Eur Respir J 2016; 44. Doi 10.1183/13993003.01384-2015.
6. Levels of Blood Periostin Decrease After Acute Myocardial Infarction and Are Negatively Associated With Ventricular Function After 3 Months. Cheng CW et al., J Investig Med, 2015; 60: 523-528.
7. Discoidin domain receptor-1 and periostin: new players in chronic kidney disease. Alfieri C et al, Nephrol Dial Transplant, 2015; 30: 1965-1971.
8. Overexpression of periostin in stroma positively associated with aggressive prostate cancer. Tian Y et al., PLoS One, 2015; 17; 10(3):e0121502.
9. Role of periostin in cancer progression and metastasis: inhibition of breast cancer progression and metastasis by anti-periostin antibody in a murine model. Int J Mol Med, 2011; 28(2):181-186.
10. Tissue expression and serum levels of periostin during pregnancy: A new biomarker of embryo-endometrial cross talk at implantation. Morelli M et al., PLoS One, 2015; 17;10(3):e0121502.

SYMBOLS

Expiry date / Verfallsdatum / Date de péremption / Data di scadenza /Fecha de caducidad / Data de validade / Uiterste gebruiksdatum / Udløbsdato / Utgångsdatum / Termin Ważności / Lejárati idő / Doba exspirácie / Doba exspirace

Consider instructions for use / Bitte Gebrauchsanweisung beachten / Consultez la notice d'utilisation / Consultare le istruzioni per l'uso / Consulte las instrucciones de utilización / Consulte as instruções de utilização / Raadpleeg de gebruiksaanwijzing / Se brugsanvisningen / Läs anvisningarna före användning / Proszę przeczytać instrukcję wykonania / Vegyük figyelembe a használati utasításban foglaltakat / Postupujte podľa pokynov na použitie / Postupujte dle návodu k použití

Lot-Batch Number / Charge-Chargennummer / Lot-Code du lot / Lotto-Numero di lotto / Lote-Código de lote / Lote-Código do lote / Lot-Partijnummer / Lot-Batchkode / Lot-Satskod / Numer serii / Lot-Batch szám / Číslo šarže / Číslo šarže

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Store at between / Lagerung bei zwischen / Conserver à entre / Conservare a tra / Conservar a temp. entre / Armazene a entre / Bewaar bij tussen / Opbevares mellem / Förvaras vid / Przechowywać w / Tároljuk …… között / Skladujte v rozsahu / Skladujte v rozmezí

Contains sufficient for x tests / Inhalt ausreichend für x Tests / Contient suffisant pour x tests / Contenuto sufficiente per x test / Contiene suficiente para x pruebas / Contém suficiente para x testes / Bevat voldoende voor x bepalingen / Indeholder tilstrækkeligt til x prøver / Innehållet räcker till x analyser / Zawartość na x testów / Tartalma X teszt elvégzésére elegendő / Obsahuje materiál pre x testov / Obsahuje materiál pro x testů

BI-20433MS MOUSE PERIOSTIN

PREPARATION OF REAGENTS:

- ❑ Bring all reagents to room temperature (18-26°C).
- ❑ Prepare reagents and samples as instructed.
- ❑ Bring unused and prepared components to the storage temperature mentioned in the package insert.
- ❑ Take microtiter strips out of the aluminium bag and mark positions on the protocol sheet.

TEST PROCEDURE:

- ❑ Step 1) Pipette 100 µl of pre-diluted (1+200) STD/SAMPLE/CTRL (Standard/Sample/Control) *(see 5) reagents and sample preparation)* in duplicate into respective wells.
- ❑ Step 2) Add 50 µl AB (biotinylated anti-periostin antibody, green cap) into each well, swirl gently.
- ❑ **Step 3) Cover tightly and incubate for 3 hours at room temperature (18-26°C).**
- ❑ Step 4) Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer, natural cap). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
- ❑ Step 5) Add 150 µl CONJ (Conjugate, amber cap) into each well, swirl gently.
- ❑ **Step 6) Cover tightly and incubate for 1 hour at room temperature (18-26°C).**
- ❑ Step 7) Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
- ❑ Step 8) Add 150 µl SUB (Substrate, blue cap) into each well, swirl gently.
- ❑ Step 9) **Incubate for 30 minutes at room temperature (18-26°C) in the dark.**
- ❑ Step 10) Add 50 µl STOP (Stop solution, white cap) into each well, swirl gently.
- ❑ Step 11) Measure absorbance immediately at 450 nm (with reference 630 nm, if available).