PERIOSTIN

(EN) ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF PERIOSTIN IN HUMAN SERUM, EDTA PLASMA, HEPARIN PLASMA OR CITRATE PLASMA
CAT. NO. BI-20433 . 12 X 8 TESTS

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

rev.no. 190109 (replacing 161201)

This kit was developed and manufactured by:
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CONTENT / INHALT

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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:

- Osteology
- Tissue repair
- Cardiovascular diseases
- Respiratory Diseases
- Oncology

2) CONTENT OF THE KIT

CONT	KIT COMPONENTS	QUANTITY
PLATE	Mouse monoclonal anti-human periostin antibody, pre-coated microtiter strips in a stripholder, packed in an aluminium bag with desiccant	12 x 8 tests
WASHBUF	Wash buffer concentrate 20x, natural cap	1 x 50 ml
AB	Goat polyclonal anti-human periostin antibody, biotinylated, green cap, ready to use	1 x 18 ml
STD	Standards 1-7, (0, 125, 250, 500, 1000, 2000, 4000 pmol/l), white caps , lyophilised	7 vials
CTRL	Control A and B, yellow cap, lyophilised (for concentration see label)	2 vials
ASYBUF	Assay Buffer, red cap, ready to use	1 x 55 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

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

4) MATERIAL AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

- Precision pipettes calibrated to deliver 10 µl, 50 µl, 150 µl, 200 µl, 300 µl, 500 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 measuring absorbance at 450 nm (reference wavelength 630 nm)
- Graph paper or software for calculation of results

5) REAGENTS AND SAMPLE PREPARATION

All reagents of the kit are stable at 4°C (2-8°C) until expiry date stated on the label of each reagent.

Sample preparation:

Collect venous blood samples by using standardized blood collection tubes for serum or plasma. We recommend performing plasma or serum separation by centrifugation as soon as possible, e.g. 10 min at 2000 x g, preferably at 4°C (2-8°C). The acquired plasma or serum samples should be measured as soon as possible. For longer storage aliquot samples and store at -25°C or lower. Do not freeze-thaw samples more than 4 times. Lipemic or haemolysed samples may give erroneous results. Samples should be mixed well before assaying.

Samples must be diluted 1+50 with assay buffer (ASYBUF), e.g. 10 µl sample + 500 µl ASYBUF.
150 µl pre-diluted sample is needed / well.

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.

Reconstitution/Handling:

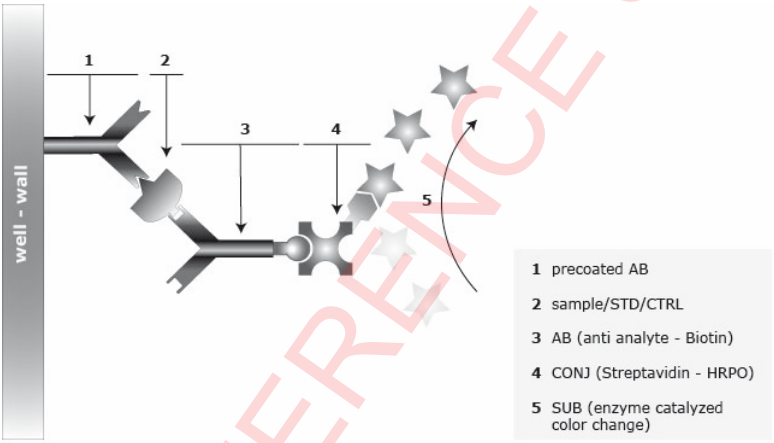
STD (Standards) and CTRL (Controls): Pipette 200 µl of distilled or deionised water into each vial. Leave at room temperature (18-26°C) for 20 min. Reconstituted STD and CTRL are stable at -25°C or lower until expiry date on label. Reconstituted STD/CTRL can undergo 1 freeze-thaw cycle.

STD / CTRL must be diluted 1+50 with assay buffer (ASYBUF), e.g. 10 µl STD / CTRL + 500 µl ASYBUF. 150 µl pre-diluted STD / CTRL is needed / well.

WASHBUF (Wash buffer): precipitation in the wash buffer concentrate may occur at lower temperatures. Dissolve precipitate by mixing gently at room temperature then dilute the concentrate 1:20 with distilled water, e.g. 50 ml WASHBUF + 950 ml distilled water, prior to use in the assay. Undiluted wash buffer is stable at 4°C (2-8°C) until expiry date on the label. Diluted wash buffer is stable at 4°C (2-8°C) up to one month. Use only diluted WASHBUF (Wash buffer) for assay performance.

6) PRINCIPLE OF THE ASSAY

This kit is a sandwich enzyme immunoassay for the quantitative determination of human Periostin in human serum and plasma samples.



7) ASSAY PROTOCOL

All reagents and samples must be at room temperature (18-26°C) before use in the assay.
Mark position for STD/ CTRL/ SAMPLE (Standard/ Control /Sample) 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.

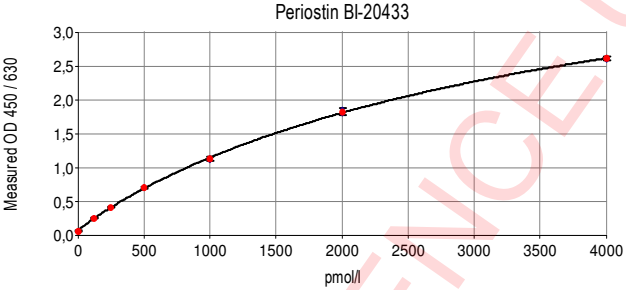
1) Add 150 µl **pre-diluted (1+50)** STD/CTRL/SAMPLE (see chapter *5) reagents and sample preparation*) into each well, swirl gently.
2) Cover tightly and incubate for 2 hours at room temperature (18-26°C).
3) Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
4) Add 150 µl AB (biotinylated anti periostin antibody, green cap) into each well, swirl gently.
5) **Cover tightly and incubate for 2 hours at room temperature (18-26°C).**
6) 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.

7) Add 150 µl CONJ (Conjugate, amber cap) into each well, swirl gently.
8) Cover tightly and incubate for 1 hour at room temperature (18-26°C).
9) 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.
10) Add 150 µl SUB (Substrate, blue cap) into each well, swirl gently.
11) Incubate for 30 min at room temperature (18-26°C) in the dark.
12) Add 50 µl STOP (Stop solution, white cap) into each well, swirl gently.
13) 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 4PL algorithm. Different curve fitting methods need to be evaluated by the user. Sample dilutions above 1+50 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 at production date. 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 1.50 or higher is obtained for the standard with the highest concentration and the values of the CTRLs are in range (target ranges see labels).

9) ASSAY CHARACTERISTICS

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)
Conversion factor	1 pg/ml = 0.011 pmol/l (MW: 91 kD)
Sample volume	150 µl (pre-diluted sample) / well
Incubation time	2 h / 2 h / 1 h / 30 min
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 concentrations of recombinant Periostin)	Serum (n=7): 106%; 95%	Heparin plasma (n=7): 92%; 85%		
	EDTA plasma (n=8): 98%; 83%	Citrate plasma (n=8): 102%; 91%		
Dilution linearity of endogenous Periostin (samples pre-diluted 1+50 according to IFU)	Average % of 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 from 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 Each laboratory should establish its own reference range for the samples under investigation. Do not change sample type during the study.			

For further information on assay 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 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
Mean (pmol/l)	249	2008
SD (pmol/l)	7.3	52
CV (%)	3	3

Inter-assay (n=12)	Sample 1	Sample 2
Mean (pmol/l)	251	1996
SD (pmol/l)	11.2	111.5
CV (%)	4	6

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 test components of human source were tested against HIV-Ab, HCV-Ab and HBsAg; and were found negative. Nevertheless, they should be handled and disposed as if they were infectious.
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 jacket while performing this assay.
- Sulfuric acid is irritating to eyes and skin. Avoid contact with skin and mucous. Irritations are possible. – Flush with water if contact occurs!

13) LITERATURE

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

In vitro Diagnostic Medical Device (for in Vitro Diagnostic Use)/ In vitro Diagnostikum (zur In-vitro-Diagnostik) / Dispositif médical de diagnostic in vitro (Pour usage diagnostique in vitro) / Dispositivo medico per diagnostica in vitro (per uso diagnostico in vitro) / Dispositivo médico de diagnóstico in vitro (para uso diagnóstico in vitro) / Dispositivo médico para diagnóstico in vitro (Para utilização de diagnóstico "in vitro") / Medisch hulpmiddel voor diagnostiek in vitro (Voor diagnostisch gebruik in vitro) / Medicinsk udstyr til in vitro-diagnostik (Udelukkende til in vitro diagnostisk anvendelse) / Medicinteknisk produkt avsedd för in vitro-diagnostik (För in vitro-diagnostiskt bruk) / Wyrób medyczny do Diagnostyki In Vitro / In vitro orvosdiagnosztikai termék / In vitro diagnostický zdravotnícky materiál (určené pre diagnostiku „in vitro“) / In vitro diagnostický zdravotnicky materiál (určeno pro diagnostiku „in vitro“)

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

Manufactured by / Hergestellt von / Fabriqué par / Prodotto da / Fabricado por / Fabricado por / Vervaardigd door / Fabrikation af / Tillverkad av / Wyprodukowane pr / Gyártotta / Vyrobené / Vyrobeno

Catalogue Number / Bestellnummer / Numéro de référence / Numero di riferimento / Número de referencia / Número de referência / Referentienummer / Referencenummer / Katalognummer / Numer katalogowy / Katalógusszám / Katalógové číslo / Katalogové číslo

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-20433 PERIOSTIN

ASSAY PROTOCOL AND CHECKLIST

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) Add 150 µl **pre-diluted (1+50)** STD/CTRL/SAMPLE (see *5) Reagents and sample preparation*) into each well. Swirl gently.
- ❑ **Step 2) Cover tightly and incubate for 2 hours at room temperature (18-26°C).**
- ❑ Step 3) 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 4) Add 150 µl AB (biotinylated anti periostin antibody, green cap) into each well. Swirl gently.
- ❑ **Step 5) Cover tightly and incubate for 2 hours at room temperature (18-26°C).**
- ❑ Step 6) Aspirate and wash wells with 300 µl WASHBUF (Wash buffer) five times. Remove remaining buffer by hitting plate against paper towel.
- ❑ Step 7) Add 150 µl CONJ (Conjugate, amber cap) into each well. Swirl gently.
- ❑ **Step 8) Cover tightly and incubate for 1 h at room temperature (18-26°C).**
- ❑ Step 9) Aspirate and wash wells with 300 µl WASHBUF (Wash buffer) five times. Remove remaining buffer by strongly tapping plate against paper towel.
- ❑ Step 10) Add 150 µl SUB (Substrate, blue cap) into each well.
- ❑ **Step 11) Incubate for 30 minutes at room temperature (18-26°C), in the dark.**
- ❑ Step 12) Add 50 µl STOP (Stop solution, white cap) into each well, swirl gently.
- ❑ Step 13) Read Optical Density at 450 nm with reference 630 nm, if available.