# *FluoBolt™-PERIOSTIN*

# *METAL ENHANCED FLUORESCENCE IMMUNOASSAY for human PERIOSTIN*

METAL ENHANCED FLUORESCENCE IMMUNOASSAY FOR
THE QUANTITATIVE DETERMINATION OF
PERIOSTIN IN HUMAN SERUM

CAT. NO. FIA-1703-F,-C3,-C5,-A6
96 Well Formate

FOR RESEARCH USE ONLY

NOT FOR USE IN DIAGNOSTIC PROCEDURES

rev.no. 190923

FIANOSTICS GmbH, A-2700 Wiener Neustadt, Viktor Kaplan Strasse 2

Tel. + 43/2622/27514, E-mail office@fianostics.at

CONTENT



FIANOSTICS
Fluorescence Immunoassays

www.fianostics.at



## 1) METAL / PLASMON ENHANCED FLUORESCENCE

Metal Enhanced Fluorescence (MEF) offers the possibility to increase the analytical sensitivity of systems based on fluorescence detection dramatically. MEF is based on the fact that excitation light interacts with the electrons of metal nano-structures thus generating very high electromagnetic fields (Localised Surface Plasmons, LSPs) Therefore, such structures are also called "plasmonic structures" and the combination of (e.g. polymeric) support and structure is known as "plasmonic substrate". These LSPs lead to an increase in emission output of fluorescent molecules (e.g. fluorescently labeled antibodies) when bound to surfaces with suitable nano-metal structures that enhances the signal dramatically. FIANOSTICS has developed a new plasmonic enhanced immunoassay platform in cooperation with Sony DADC BioSciences (now STRATEC Consumables since July 1st 2016), that allows up to 300 fold gains of sensitivity. This platform is fully compatible to standard laboratory methodology using 96 well microtiter plate format and assays based on this technology can be run on any standard fluorescence microplate reader. Its unique features enable fluorescence immunoassays with highest sensitivity and without washing steps.

## 2) PERIOSTIN

PERIOSTIN (UniProtKB - Q15063), also known as osteoblast specific factor 2 (OSF-2), is a cell adhesion protein belonging to the fasciclin domain-containing protein family. It consists of 836 amino acids (aa) starting with a 21 aa long signalling sequence, followed by an Emilin-like domain rich in cysteine, four repeated fasiclin1 and a C terminal variable domain, which is different among the 7 splice variants (isoforms) in humans.

PERIOSTIN is expressed during ontogenesis as well as in adult connective tissues submitted to mechanical stress such as bone, tendons, heart valves, skin and the periodontal ligaments. Further, it is expressed in aorta, stomach, lower gastrointestinal tract, placenta, uterus, thyroid and breast tissue. In bone, PERIOSTIN directly interacts with collagen type I, fibronectin, Notch 1, tenascin-C and BMP-1, resulting in enhanced proteolytic activation of lysyl oxidase for collagen cross-linking stabilising bone matrix. Next to developing, maintaining and repairing tissue, PERIOSTIN plays a vital role in tumorigenesis by interacting with various cell-surface receptors and signaling pathways, which e.g. results in inactivation of integrin-mediated signaling, leading to promoting cell adhesion and motility which is of relevance for tumor progression and metastasis. Research has investigated association of elevated serum PERIOSTIN levels with pancreatic, ovarian, lung, breast, colon, gastric, thyroid and oesophageal tumours.

## 3) CONTENT OF THE KIT

| ID | KIT COMPONENT | QUANTITY |
|---|---|---|
| FM | Anti-human PERIOSTIN antibody, pre-coated MEF-microtiter plate, packed in vacuum sealed aluminium bag | 1 x 96 well |
| WP | Wash buffer concentrate 20x, natural cap | 1 x 25 ml |
| FAF, FA3, FA5, FAA | Anti-human PERIOSTIN antibody, black flask, labeled with FITC, Cy3, Cy5 or AlexaFluor680 | 1 x 4 ml |
| FS | Standards 1-6, (0, 11.25, 22.5, 45, 90, 180 pmol/l), white caps, lyophilized | 6 vials, 0.25 ml |
| FCA/B | Control A and B, yellow cap, lyophilized (for concentrations see label) | 2 vials, 0.25 ml |
| FD | Sample diluent, natural cap, ready to use | 1 x 10 ml |

## 4) ADDITIONAL MATERIAL SUPPLIED WITH THE KIT

- 2 self-adhesive plastic films



- QC data sheet
- Protocol sheet
- Instruction manual for use
- 2 desiccant bags for plate storage

## 5) MATERIAL AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

- Precision pipettes calibrated to deliver 10 µl, 20 µl, 50 µl, 200 µl, 500 µl and disposable tips
- Distilled or deionized water
- Plate washer, multichannel pipette or manifold dispenser for washing
- Refrigerator with 4°C (2-8°C)
- Fluorescence microplate reader
- Graph paper or software for calculation of results

## 6) 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:

Adding recombinant PERIOSTIN to serum and plasma resulted in 25% recovery in EDTA/Heparin-plasma and 80% in Citrate-plasma compared to serum (100%). This may be a result of the heparin and/or fibronectin binding properties of this molecule. Therefore, we suggest to use only serum samples for optimal results.

Collect venous blood samples by using standardized blood collection tubes for serum. We recommend performing 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 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.
For further information on sample stability contact us by e-mail at support@fianostics.at or by phone + 43/2622/27514.

Reagent preparation:

Add 250 µl of distilled or deionized water to the lyophilized FS (Standards) and FC (Controls). Leave at room temperature (18-26°C) for 20 min. Reconstituted FS and FC are stable at -25°C or lower until expiry date stated on the label. Reconstituted FS and FC can undergo 4 freeze-thaw cycles.
Bring WP (Wash buffer) concentrate (20x) to room temperature. Make sure that the solution is clear and without any salt precipitates before further dilution. Dilute the WP to working strength by adding the appropriate amount of distilled or deionized water, e.g. 25 ml of WP + 475 ml water, prior to use in the assay. Undiluted WP is stable at 4°C (2-8°C) until expiry date on the label. Diluted WP is stable at 4°C (2-8°C) up to one month. Only use diluted WP in the assay.

**7) ASSAY PROCEDURE**

All reagents and samples must be at room temperature (18-26°C) before use in the assay.
Mark position for standards, controls and samples on the protocol sheet. We recommend to run samples and standards in duplicates.



Take the plasmonic enhanced microtiter plate out of the aluminium bag. Avoid touching the bottom of the plate with bare hands, because reading without washing is performed through the well bottom.

In standard format, the kit is delivered with an AlexaFluor680 labeled detection antibody (FAA) because serum background fluorescence is minimal within this wavelength range. Therefore, if your reader is equipped with monochromatic optics, please set Excitation/Emission to 679/702 nm or if you are using an optical filter based reader, select a suitable filter pair (e.g. 670/720 nm). On request the kit can also be delivered with FITC, Cy3 or Cy5 (Ex/Em = 495/518 nm, 550/570 nm or 650/670 nm) labeled detection antibody.

1) Add 40 µl of the selected fluorescence labeled detection antibody (FAF or FA3 or FA5 or FAA) to all wells required. Swirl gently.

2) Add 20 µl of standard, control or sample to the wells according to the marked positions on the protocol sheet, swirl gently, cover tightly with the delivered adhesive film and incubate over night at 37°C in the dark.

3a) If your reader allows bottom reading, read the plate without any further processing at the Ex/Em wavelength fitting to the delivered detection antibody (495/518 nm for FAF, 550/570 nm for FA3, 650/670 nm for FA5, 679/702 nm for FAA). Gain should be set to achieve at least 10000 fluorescence units (F.U.) between the signal of the 0 pM and the 180 pM PERIOSTIN standard. Samples with signals exceeding the signal of the highest standard must be re-run with an appropriate dilution using sample diluent (FD).

3b) If your reader has no bottom read option or if you want to store the plate for documentation purposes, discard or aspirate the content of the wells and wash 3x with diluted wash buffer.

Use a minimum of 200 µl wash buffer per well. After the final wash, remove remaining fluid by strongly tapping the plate against a paper towel. Read the plate in top configuration without any further processing at the Ex/Em wavelength fitting to the chosen detection antibody (495/518 nm for FAF, 550/570 nm for FA3, 650/670 nm for FA5, 679/702 nm for FAA).

**Hint: Quality of bottom reading (3a) may vary between microplate readers. For first time users we suggest to perform the washing step and follow protocol 3b.**

Gain should be set to achieve at least 10000 fluorescence units (F.U.) between the signals of the 0 pM and the 180 pM PERIOSTIN standard. Samples with signals exceeding the signal of the highest standard must be re-run with appropriate dilution using sample diluent (FD).

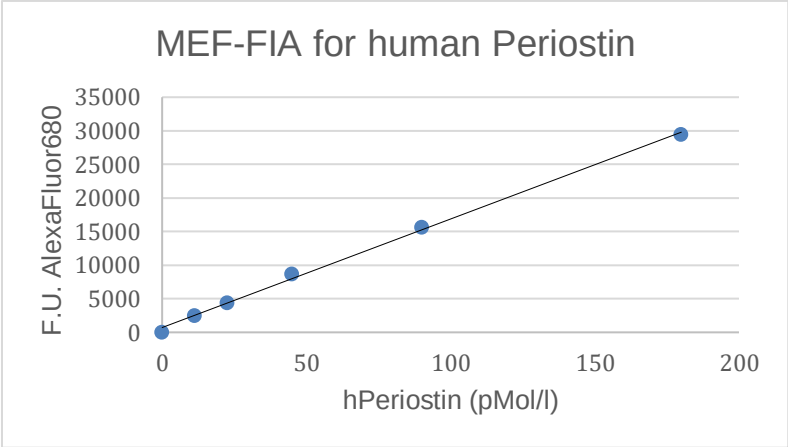
4) Store the plate with the 2 desiccant bags supplied at 4°C (2-8°C) in the aluminium bag. Unused wells are stable until expiry date stated on the label. Fluorescence signals of standards, controls and samples remain detectable for at least two month at the plate surface, depending on signal intensity achieved.

**8) CALCULATION OF RESULTS**

Subtract the fluorescence intensity of the 0 pM standard from all other standards, samples and controls. Construct a calibration curve from the fluorescence units (F.U.) of the standards using commercially available software or graph paper. Read sample and control concentrations from this standard curve. Make sure to use appropriate curve fitting algorithm (e.g. linear or 4PL)



Example of a typical calibration 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.

Fluorescence intensity 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 the supplied kit controls read according to specifications (target ranges see labels).

## 9) ASSAY CHARACTERISTICS

| | |
|---|---|
| Method | Metal Enhanced Direct Sandwich Fluorescence Immunoassay in 96-well plate format |
| Sample type | Serum |
| Standard range | 0 to 180 pmol/l (6 standards and 2 controls in a serum based matrix) |
| Conversion factor | 1 ng/ml = 11 pmol/l (MW: 93.3 kD) |
| Sample volume | 20 µl (undiluted sample) / well |
| Incubation time / | over night / 37°C |
| Sensitivity | LOD (0 pmol/l + 3 SD): 2 pmol/l; LLOQ: 11 pmol/l |
| Specificity | This assay detects human PERIOSTIN. No interference of BMP-1 or TGF-ß1 with the assay's signal up to a 10 fold molar excess was monitored. |
| Cross-reactivity | Human PERIOSTIN shares around 99-98% aa sequence identity with higher apes, 95% with bovine/ equine but only 91% with mouse PERIOSTIN. Cross reactivity of this assay with other species than human has not been tested. |

Precision:

Intra-assay: 4 samples of known concentrations were tested 4 times within 1 assay run
CVs ranged from 3.5-7.5%

Inter-assay: 4 samples of known concentrations were tested in duplicates within 3 different assay runs
CVs ranged from 9-13%

Spike/Recovery:

The recovery of PERIOSTIN was evaluated by adding known amounts of human recombinant PERIOSTIN to 4 different human serum samples.
Mean recovery was 82% (71-100%)



Specificity:

Analyte Specificity:

This assay detects human PERIOSTIN. Addition of recombinant TGF-β1 or BMP-1, which are considered to be binding to PERIOSTIN, to the standards supplied with this kit did not reduce signal intensity.

Species Specificity:

Human PERIOSTIN shares around 98-99% aa sequence identity with higher apes (e.g. gorilla or chimpanzee), 95% with bovine/ equine and 91% with mouse PERIOSTIN. Reactivity of this assay with other species than human has not been tested. So using this assay for PERIOSTIN measurements in serum of species with high sequence homology may be possible, but must be evaluated by the user. FIANOSTICS does not take responsibility for functionality of the assay in non-human samples

## 10) 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.

## 11) PRECAUTIONS

- All test components of human source were tested against HIV-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 300 as preservative. Proclin 300 is not toxic in concentrations used in this kit. It may cause allergic skin reactions – avoid contact with skin, eyes or mucous membranes.
- Do not pipette by mouth.
- Do not eat, drink, smoke or apply cosmetics where reagents are used.
- Wear gloves, protective glasses and lab jacket while performing this assay.
- For Research Use Only. Not for Diagnostic Purposes.

## 12) LITERATURE


- **Metal-enhanced fluorescence: an emerging tool in biotechnology.** Aslan K, Gryczynski I, Malicka J, Matveeva E, Lakowicz JR, Geddes CD. Curr Opin Biotechnol, 2005; 16(1): 55-62.

- **Periostin action in bone**. Bonnet N, Garnero P, Ferrari S, Mol Cell Endocrinol. 2016; 5; 432:75-82.

- **Serum periostin levels serve as a biomarker for both eosinophilic airway inflammation and fixed airflow limitation in well-controlled asthmatics**. Takahashi K. et al., J Asthma. 2018; 12:1-8.





- **Serum periostin during omalizumab therapy in asthma: A tool for patient selection and treatment evaluation.** Caminati M, et al. Ann Allergy Asthma Immunol. 2017; 119 (5):460-462.

- P**eriostin: A Matricellular Protein With Multiple Functions in Cancer Development and Progression.**González-González L, Alonso J.Front Oncol. 2018; 12; 8:225.

- **Epithelial periostin expression is correlated with poor survival in patients with invasive breast carcinoma.** Kim GE, PLoS One., 2017; 12(11)

- **Influence of Periostin on Synoviocytes in Knee Osteoarthritis**. Tajika Y. et al., In Vivo. 2017; 31 (1):69-77.

- **Serum periostin levels following small bone fractures, long bone fractures and joint replacements: an observational study**. Varughese R. et al., Allergy Asthma Clin Immunol. 2018; 14:30

## 13) NOTES

This page is left blank intentionally to be used for your personal notes regarding the assay.