Product information

Useŕs Manual

Periostin human ELISA

Enzyme immunoassay (ELISA) for the determination of human periostin in cell culture supernatants, serum and plasma.

REF IB99515

Σ 96

Storage: 2-8°C

RUO **For research use only, not for use in diagnostic procedures.**

Version 2 (May-12-2016)

Manufactured for:
Immuno-Biological Laboratories, Inc. (IBL-America)
8201 Central Ave NE, Suite P, Minneapolis, MN 55432
Toll Free: (888) 523-1246 Fax: (763) 780-2988
www.ibl-america.com / info@ibl-america.com

Table of Contents

1. Intended Use

The Periostin human ELISA kit is to be used for the measurement of human periostin in cell culture supernatants, serum and plasma. This ELISA kit is for research use only.

2. Introduction

Periostin, also termed osteoblast-specific factor 2 (OSF-2), is a 90-kDa secreted protein that shares a homology with the insect axon guidance protein fasciclin I (1). Periostin is one of four known mammalian genes that contain fasciclin domains with stabilin 1 and 2, as well as TGFβ-Induced Gene-Human clone 3 (BIGH3) (2).

Periostin protein is composed of a signal sequence, four-coiled fasciclin-like repeats, an amino-terminal cysteine-rich region (EMI domain), and heparin-binding domains present in the carboxyl tail. Periostin contains gamma-carboxyglutamate residues that are formed by vitamin K dependent carboxylation (3). These residues are essential for the binding of calcium. Periostin is thought to be involved in osteoblast recruitment, attachment and spreading. It is a component of the extracellular matrix

The N-terminus part of periostin (up to exon 16) is conserved, while the C-terminal region (comprising exon 17–23) gives rise to different splice isoforms upon alternative splicing. The isoforms have a molecular weight range from 83 kDa to 93 kDa (4). Six different periostin splice isoforms have been reported, but only four of them were sequenced and annotated.

Periostin is expressed during ontogenesis and down regulated in adult except in bones, in collagen-rich fibrous connective tissues subjected to constant mechanical stress, such as periodontal ligament (PDL), heart valves, skin and tendons. Periostin expression is also observed in niches in direct contact with tissue-specific stem cells in mammary gland, bone, and intestine. Periostin has been found to be overexpressed in various type of human tumors including neuroblastoma, head and neck cancer, nasopharyngeal carcinoma, non-small cell lung carcinoma, breast cancer, colon cancer, pancratic ductal adenocarcinoma and ovarian cancer (5). Isoforms of periostin are over-expressed by stromal cells in several human ovary, breast, colon and brain tumors.

Abnormal expression of periostin is also linked to angiogenesis and metastasis in epithelial tumors. Periostin is expressed by fibroblasts in the normal tissue and in the stroma of the primary tumour. Infiltrating tumour cells need to induce stromal Periostin expression in the secondary target organ to initiate colonization. Periostin is crucial for cancer stem cell maintenance (6). Periostin up-regulation in cancers usually correlates with aggressiveness and/or poor survival.

3. General References

(1) Periostin: novel diagnostic and therapeutic target for cancer: Y. Kudo, et al.; Histol. Histopathol. **22,** 1167 (2007)

(2) The many facets of the matricellular protein periostin during cardiac development, remodeling, and pathophysiology: A. Russel, et al.; J. Cell Commun. Signal. **3,** 275 (2009)

(3) Periostin, a member of a novel family of vitamin K-dependent proteins, is expressed by mesenchymal stromal cells: D.L. Coutu, et al.; J. Biol. Chem. **283,** 17991 (2008)

(4) Characterization of periostin isoform pattern in non-small cell lung cancer: L. Morra, et al.; Lung Cancer **76,** 183 (2012)

(5) Role of periostin in cancer progression and metastasis: inhibition of breast cancer progression and metastasis by anti-periostin antibody in a murine model: M. Kyutoku, et al.; Int. J. Mol. Med. **28,** 181 (2011)

(6) Interactions between cancer stem cells and their niche govern metastatic colonization: I. Malanchi, et al.; Nature **481,** 85 (2011)

4. Assay Principle

This assay is a sandwich Enzyme Linked-Immunosorbent Assay (ELISA) for the measurement of human periostin in cell culture supernatants, serum and plasma. A monoclonal antibody specific for periostin has been precoated onto the 96-well microtiter plate. Standards (STD) and samples are pipetted into the wells for binding to the coated antibody. After extensive washing to remove unbound compounds, periostin is recognized by the addition of a biotinylated monoclonal antibody specific for periostin (DET). After removal of excess biotinylated antibody, streptavidine-peroxidase (STREP-HRP) is added. Following a final washing, peroxidase activity is quantified using the substrate 3,3',5,5'-tetramethylbenzidine (TMB). The intensity of the color reaction is measured at 450 nm after acidification and is directly proportional to the concentration of periostin in the samples.

5. Handling & Storage

- Reagent must be stored at 2-8°C when not in use
- Plate and reagents should be at room temperature before use.
- Do not expose reagents to temperatures greater than 25°C.

6. Kit Components

• 1 vial human periostin Standard (lyophilized)	(1 µg)	(STD)
• 1 vial Detection Antibody	(20 µl)	(DET)
• 1 vial HRP Labeled Streptavidin (lyophilized)	(2 µg)	(STREP-HRP)
• 2 bottles Wash Buffer 10X	(2 x 30 ml)	(Wash Buffer 10X)
• 2 bottles ELISA Buffer 10X	(2 x 30 ml)	(ELISA Buffer 10X)
• 1 bottle TMB Substrate Solution	(12 ml)	(TMB)
• 1 bottle Stop Solution	(12 ml)	(STOP)
• 1 plate coated with periostin Antibody	(6 x 16-well strips)	
• 2 plate Covers (plastic film)		
• 2 silica Gel Minibags		

7. Materials Required but *Not* Supplied

- Microtiterplate reader at 450nm
- Calibrated precision pipettes. Disposable pipette tips
- Deionized water
- Microtubes or equivalent for preparing dilutions
- Disposable plastic containers for preparing working buffers
- Plate washer: automated or manual
- Glass or plastic tubes for diluting and aliquoting standard

8. General ELISA Protocol

8.1. Preparation and Storage of Reagents

NOTE: *Prepare just the appropriate amount of the buffers necessary for the assay.*

- **Wash Buffer 10X** has to be diluted with deionized water 1:10 before use (e.g. 30 ml Wash Buffer 10X + 270 ml water) to obtain Wash Buffer 1X.
- **ELISA Buffer 10X** has to be diluted with deionized water 1:10 before use (e.g. 10 ml ELISA Buffer 10X + 90 ml water) to obtain ELISA Buffer 1X.
- **Detection Antibody (DET)** has to be diluted to 1:1000 in ELISA Buffer 1X (2 µl DET + 2 ml ELISA Buffer 1X).

  NOTE: *The diluted Detection Antibody is not stable and cannot be stored!*

- **HRP Labeled Streptavidin (STREP-HRP)** has to be reconstituted with 100 µl of ELISA Buffer 1X.
  - After reconstitution of STREP-HRP, prepare aliquots and store them at -20°C. **Avoid freeze/thaw cycles**.
  - Dilute the reconstituted STREP-HRP to the working concentration by adding 50 µl in 10 ml of ELISA Buffer 1X (1:200).

  NOTE: *The diluted STREP-HRP is not stable and cannot be stored!*

- **Human periostin Standard (STD)** has to be reconstituted with 100 µl of ELISA Buffer 1X.
  - This reconstitution produces a stock solution of 10 µg/ml. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes **at 37°C.** Mix well prior to making dilutions.

  NOTE: *The reconstituted standard is aliquoted and stored at -20°C!*

  - Dilute the standard protein concentrate (STD) (**10 µg/ml**) in ELISA Buffer 1X. A seven-point standard curve using 2-fold serial dilutions in ELISA Buffer 1X is recommended.
  - Suggested standard points are:

    5000, 2500, 1250, 625, 312, 156, 78, and 0 pg/ml.

Start with the dilution of the concentrate (STD):

To obtain	Add	Into
100 ng/ml	10µl of periostin (STD) (10 µg/ml)	990 µl of ELISA Buffer 1X

Dilute further for the standard curve:

To obtain	Add	Into
5000 pg/ml	50 µl of periostin (100 ng/ml)	950 µl of ELISA Buffer 1X
2500 pg/ml	300 µl of periostin (5000 pg/ml)	300 µl of ELISA Buffer 1X
1250 pg/ml	300 µl of periostin (2500 pg/ml)	300 µl of ELISA Buffer 1X
625 pg/ml	300 µl of periostin (1250 pg/ml)	300 µl of ELISA Buffer 1X
312 pg/ml	300 µl of periostin (625 pg/ml)	300 µl of ELISA Buffer 1X
156 pg/ml	300 µl of periostin (312 pg/ml)	300 µl of ELISA Buffer 1X
78 pg/ml	300 µl of periostin (156 pg/ml)	300 µl of ELISA Buffer 1X
0 pg/ml	300 µl of ELISA Buffer 1X	Empty tube

8.2. Sample collection, storage and dilution

Cell Culture Supernatants, serum and plasma have to be diluted in ELISA Buffer 1X. Starting dilutions of 1/50 to 1/100 are recommended.

8.3. Assay Procedure (Checklist)

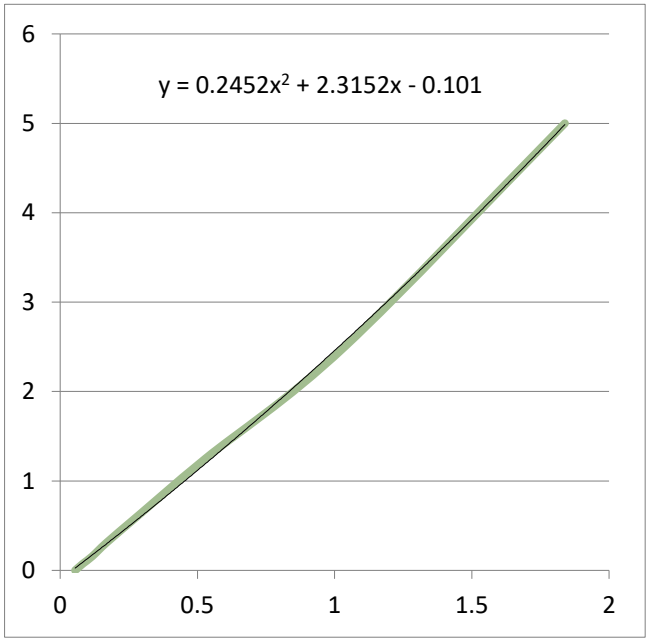
☐	1. Determine the number of 16-well strips needed for the assay and insert them in the frame for current use. The extra strips are left in the bag with 2 silica gel minibags and stored at 4°C. **NOTE:** *Remaining 16-well strips coated with* periostin *antibody when opened can be stored in the presence of 2 silica gel minibags at 4°C for up to 1 month.*
☐	2. Add 100 µl of the different standards into the appropriate wells in duplicate! At the same time, add 100 µl of diluted serum, plasma or cell culture supernatant samples in duplicate to the wells **(see 8.1. Preparation and Storage of Reagents and 8.2 Preparation of Samples).**
☐	3. Cover the plate with plastic film and incubate for **2 h at 37°C.**
☐	4. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of five washes. After the last wash, complete removal of liquid is essential for good performance.
☐	5. Add 100 µl to each well of the diluted Detection Antibody **(DET) (see 8.1 Preparation and Storage of Reagents)**.
☐	6. Cover the plate with plastic film and incubate for **1 h at 37°C**.
☐	7. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of five washes. After the last wash, complete removal of liquid is essential for good performance.
☐	8. Add 100 µl to each well of the diluted HRP Labeled Streptavidin **(STREP-HRP) (see 8.1. Preparation and Storage of Reagents)**.
☐	9. Cover the plate with plastic film and incubate for **30 min at RT**.
☐	10. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of five washes. After the last wash, complete removal of liquid is essential for good performance.
☐	11. Add 100 µl to each well of TMB substrate solution **(TMB)**.
☐	12. Allow the color reaction to develop **at RT in the dark for 10-20 minutes**. Do not cover the plate.
☐	13. Stop the reaction by adding 50 µl of Stop Solution (**STOP**). Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue solution that turns yellow when Stop Solution (**STOP**) is added.
	! CAUTION: CORROSIVE SOLUTION !
☐	14. Measure the OD at 450 nm in an ELISA reader.

9. Calculation of Results

- Average the duplicate readings for each standard, control and sample and subtract the average blank value (obtained with the 0 pg/ml point).
- Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the horizontal (X) axis vs. the corresponding periostin concentration (pg/ml) on the vertical axis (see **10.** TYPICAL DATA).
- Calculate the periostin concentrations of samples by interpolation of the regression curve formula as shown above in a form of a quadratic equation
- If the test sample was diluted, multiply the interpolated value by the dilution factor to calculate the concentration of human periostin in the sample.

10. Typical Data

The following data are obtained using the different concentrations of standard as described in this protocol:



Standard periostin (pg/ml)	Optical Density (mean)
5000	2.11
2500	1.19
1250	0.60
625	0.33
312.5	0.195
156	0.133
78	0.096
0	0.062

Figure: Standard curve

11. Performance Characteristics

A. Sensitivity (Limit of detection):

The lowest level of periostin that can be detected by this assay is 15 pg/ml. **NOTE:** *The Limit of detection was measured by adding three standard deviations to the mean value of 50 zero standard.*

B. Assay range: 78 pg/ml – 5000 pg/ml

C. Specificity:

This ELISA is specific for the measurement of natural and recombinant human periostin. It has been tested on human periostin isoforms 1 and 2 (should detect human isoforms 3 and 4). It does cross-react with mouse periostin.

D. Intra-assay precision:

Four samples of known concentrations of human periostin were assayed in replicates 8 times to test precision within an assay.

Samples	Means (ng/ml)	SD	CV (%)	n
A1	18.92	1.21	6.41	8
A2	31.83	2.74	8.60	8
A3	27.51	1.99	7.23	8
A4	16.62	1.034	6.22	8

E. Inter-assay precision:

Four samples of known concentrations of human periostin were assayed in 4 separate assays to test precision between assays.

Samples	Means (ng/ml)	SD	CV (%)	n
B1	28.44	2.81	9.90	4
B2	23.99	2.36	9.85	4
B3	21.09	0.95	4.54	4
B4	27.06	1.67	6.18	4

F. Linearity:

Different samples containing human periostin were diluted several fold (1/50 to 1/100) and the measured recoveries ranged from 95% to 105%.

G. Expected values:

Human periostin levels range in plasma or serum from **10 to >100ng /ml**.

12. Technical Hints and Limitations

- It is recommended that all standards, controls and samples be run in duplicate.
- Do not combine leftover reagents with those reserved for additional wells.
- Reagents from the kit with a volume less than 100µl should be centrifuged.
- Residual wash liquid should be drained from the wells after last wash by tapping the plate on absorbent paper.
- Crystals could appear in the 10X solution due to high salt concentration in the stock solutions. Crystals are readily dissolved at room temperature or at 37°C before dilution of the buffer solutions.
- Once reagents have been added to the 16-well strips, DO NOT let the strips DRY at any time during the assay.
- Keep TMB Solution protected from light.
- The Stop Solution (STOP) consists of sulfuric acid. Although diluted, the Stop Solution should be handled with gloves, eye protection and protective clothing.

13. Troubleshooting

PROBLEM	POSSIBLE CAUSES	SOLUTIONS
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in the correct order.
	Washes too stringent	Use an automated plate washer if possible.
	Incubation times inadequate	Incubation times should be followed as indicated in the manual.
	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrates to room temperature before use.
High background	Concentration of STREP-HRP too high	Use recommended dilution factor.
	Inadequate washing	Ensure all wells are filling wash buffer and are aspirated completely.
Poor standard curve	Wells not completely aspirated	Completely aspirate wells between steps.
	Reagents poorly mixed	Be sure that reagents are thoroughly mixed.
Unexpected results	Omission of reagents	Be sure that reagents were prepared correctly and added in the correct order.
	Dilution error	Check pipetting technique and double-check calculations.

14. Notes

For orders please contact:

Immuno-Biological Laboratories, Inc. (IBL-America)
8201 Central Ave NE. Suite P
Minneapolis, MN 55432, USA
toll-free phone: +1 (888) 523 1246
phone: +1 (763) 780 2955
fax: +1 (763) 780 2988
e-mail: info@ibl-america.com
web: www.ibl-america.com