BioVendor Group

ENG

Instructions for Use:

MOUSE PERIOSTIN ELISA

Catalogue number:

RAG020R

For research use only.

BioVendor R&D

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CONTENT

HISTORY OF CHANGES

Previous version	Current version
ENG.002.A	ENG.003.A
History of changes added.	
A symbol indicating the manufacturer added.	

1. INTENDED USE

The Mouse Periostin ELISA Kit is to be used for the in vitro quantitative determination of mouse periostin in cell culture supernatants, serum and plasma. This ELISA Kit is for research use only.

2. 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.

3. 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.

4. TEST PRINCIPLE

This assay is a sandwich Enzyme Linked-Immunosorbent Assay (ELISA) for quantitative determination of mouse 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. TECHNICAL HINTS

- It is recommended that all standards, controls samples 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 100 µl 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 8-well strips, DO NOT let the strips DRY at any time during the assay.
- Keep TMB Substrate Solution protected from light.
- The Stop Solution consists of phosphoric acid. Although diluted, the Stop Solution should be handled with gloves, eye protection and protective clothing.

6. REAGENT SUPPLIED

Kit Components	Quantity
1 plate coated with periostin Antibody	6 x 16-well strips
2 bottles Wash Buffer 10X	2 x 30 ml
2 bottles ELISA Buffer 10X	2 x 30 ml
1 vial Detection Antibody	20 µl
1 vial HRP Labeled Streptavidin (lyophilized)	2 µg
1 vial mouse periostin Standard (lyophilized)	5 ng
1 bottle TMB Substrate Solution	12 ml
1 bottle Stop Solution	12 ml
2 plate sealers (plastic film)	
2 silica Gel Minibags	

7. MATERIAL REQUIRED BUT NOT SUPPLIED

- Microtiterplate reader at 450 nm
- Calibrated precision single and multi-channel 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. PREPARATION OF REAGENTS

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

8.1 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.

8.2 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.

8.3 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!

8.4 HRP Labeled Streptavidin (STREP-HRP)

has to be reconstituted with 100µl of ELISA Buffer 1x.

- After reconstitution of STREP-HRP, prepare aliquots and store them at -20°C. **Avoid freeze/thaw cycles**.
- Dilute the reconstituted STRE-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!

8.5 Mouse Periostin Standard (STD)

has to be reconstituted with 100µl of ELISA Buffer 1x.

- This reconstitution produces a stock solution of 50 µg/ml. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes at RT. Mix well prior to making dilutions.

Note: The reconstituted standard is aliquoted and stored at -20°C

- Dilute the standard protein concentrate (STD) (**50 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:

2000, 1000, 500, 250, 125, 62.5, 31.25, and 0 pg/ml.

Start with the dilution of the concentrate (STD):

To obtain	Add	Into
500 ng/ml	10µl of periostin	990 µl of ELISA Buffer 1X
20 ng/ml	20µl of periostin	480 µl of ELISA Buffer 1X

Dilute further for the standard curve:

To obtain	Add	Into
2000 pg/ml	100 µl of periostin (20 ng/ml)	900 µl of ELISA Buffer 1X
1000 pg/ml	300 µl of periostin (2000 pg/ml)	300 µl of ELISA Buffer 1X
500 pg/ml	300 µl of periostin (1000 pg/ml)	300 µl of ELISA Buffer 1X
250 pg/ml	300 µl of periostin (500 pg/ml)	300 µl of ELISA Buffer 1X
125 pg/ml	300 µl of periostin (250 pg/ml)	300 µl of ELISA Buffer 1X
62.5 pg/ml	300 µl of periostin (125 pg/ml)	300 µl of ELISA Buffer 1X
31.25 pg/ml	300 µl of periostin (62.5 pg/ml)	300 µl of ELISA Buffer 1X
0 pg/ml	300 µl of ELISA Buffer 1X	Empty tube

9. PREPARATION OF SAMPLES

Cell Culture Supernatants, serum and plasma have to be diluted in ELISA Buffer 1X. Starting dilutions of 1/4000 to 1/8000 are recommended.

10. ASSAY PROCEDURE

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 Preparation and Storage of Reagents and Preparation of Samples).
3. Cover the plate with plastic film and incubate for **2 h at RT.**
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 Preparation and Storage of Reagents).
6. Cover the plate with plastic film and incubate for **1 h at RT**.
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 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.

11. CALCULATIONS

- 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 (Y) axis (see 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 samples were diluted, multiply the interpolated values by the dilution factor to calculate the concentration of mouse periostin in the samples.

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

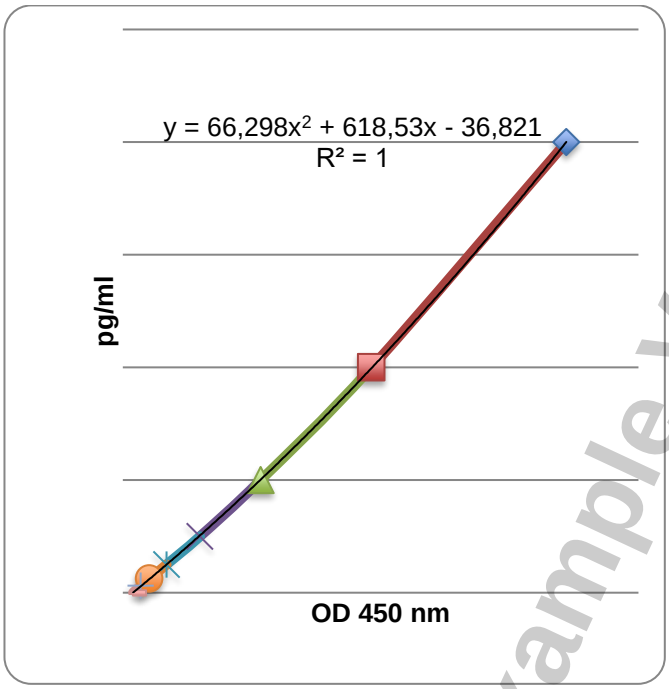


Figure: *Standard curve*

Standard periostin (pg/ml)	Optical Density (mean)
2000	2.58
1000	1.446
500	0.802
250	0.449
125	0.254
62.5	0.155
31.25	0.105
0	0.06

12. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Mouse Periostin ELISA are presented in this chapter

12.1 Sensitivity (Limit of detection)

The lowest level of periostin that can be detected by this assay is 10 pg/ml.

Note: The Limit of detection was measured by adding three standard deviations to the mean value of 50 zero standard.

12.2 Assay range

31.25 pg/ml – 2000 pg/ml

12.3 Specificity

This ELISA is specific for the measurement of natural and recombinant mouse periostin. It has been tested on mouse periostin isoforms 5 (should also detect mouse isoforms 1, 2 and 3 according to sequences). Mouse isoform 4 has not been tested.

12.4 Precision

12.4.1 Intra-assay (n =4)

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

Sample	Mean (µg/ml)	SD (µg/ml)	CV (%)
1	1.991	0.043	2.14
2	1.017	0.017	1.70
3	2.024	0.072	3.55
4	3.048	0.051	1.67

12.4.2 Inter-assay (n = 3)

Four samples of known concentrations of mouse periostin were assayed in 3 separate assays to test precision between assays.

Sample	Mean (µg/ml)	SD (µg /ml)	CV (%)
1	1.36	0.096	7.05
2	1.97	0.18	8.98
3	1.36	0.09	6.63
4	1.81	0.14	7.67

12.5 Spinking Recovery:

When samples (serum or plasma) are spiked with known concentrations of mouse periostin, the recovery averages 96% (range from 89% to 115%).

12.6 Linearity

Different samples containing mouse periostin were diluted several fold (1/4000 to 1/8000) and the measured recoveries ranged from 95% to 105%.

12.7 Expected values:

Mouse periostin levels range in plasma or serum from **1 µg to >10 µg/ml**.

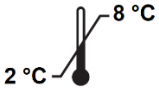
13. TROUBLESHOOTING

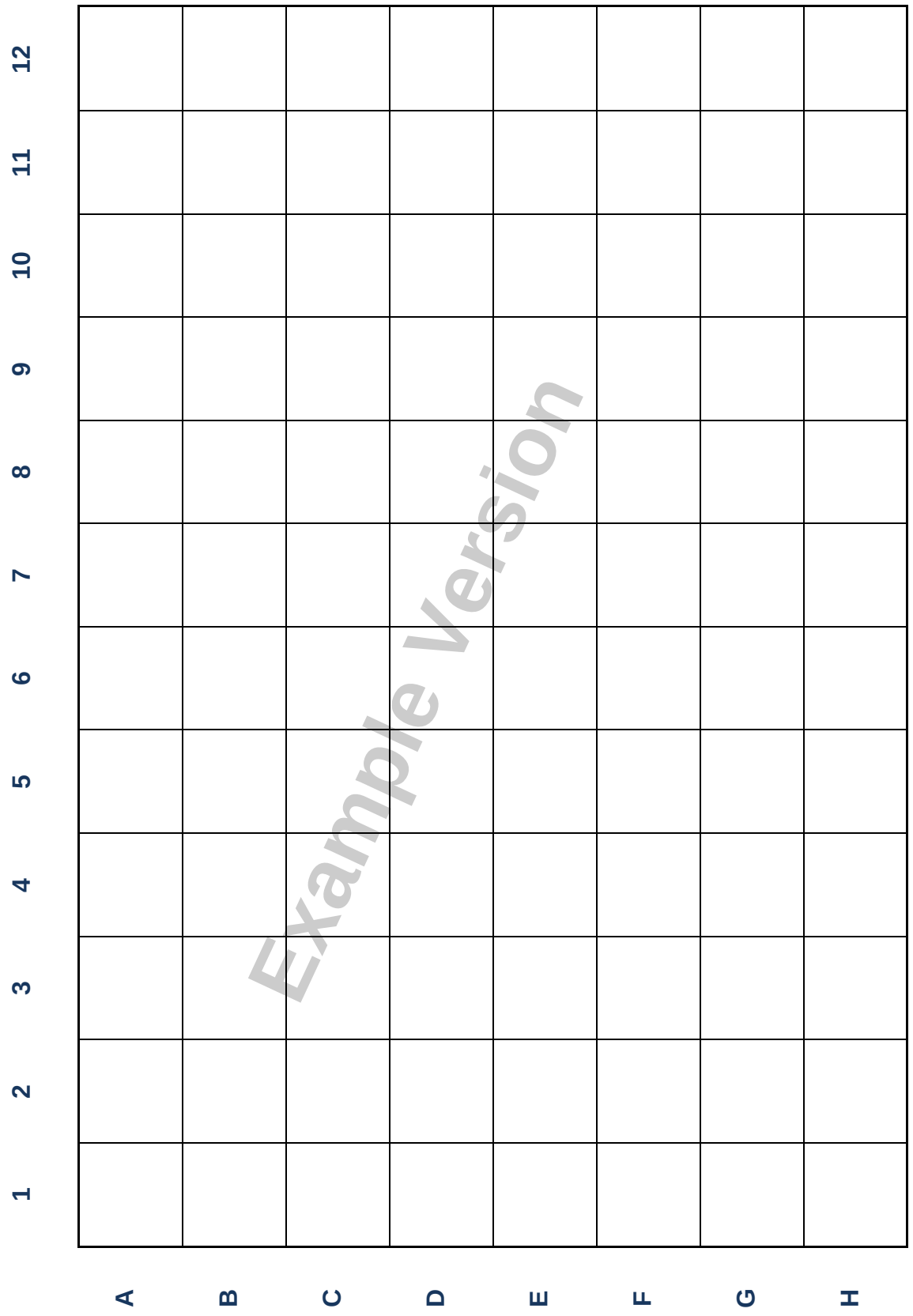
PROBLEM	POSSIBLE CAUSES	SOLUTIONS
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in the correct order.
	Washes too stringent	Use an automated plate washer if possible.
	Incubation times inadequate	Incubation times should be followed as indicated in the manual.
	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. REFERENCES

1. Periostin: novel diagnostic and therapeutic target for cancer. Kudo Y., et al. Histol Histopathol. 22:1167 (2007).
2. The many facets of the matricellular protein periostin during cardiac development, remodeling, and pathophysiology. Russel A., 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. Coutu D.L., et al. J Biol Chem. 283: 17991 (2008).
4. Characterization of periostin isoform pattern in non-small cell lung cancer. Morra L, 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. Kyutoku M., et al. Int J Mol Med. 28: 181 (2011).
6. Interactions between cancer stem cells and their niche govern metastatic colonization. Malanchi I., et al. Nature. 481 :85 (2011)

15. EXPLANATION OF SYMBOLS

	Catalogue number
	Batch code
	Caution
	Use by date
	Temperature limit
	Manufacturer
	Read electronic instructions for use - eIFU
	The content is sufficient for 96 tests
	Biological risks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Example Version

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