

Quantikine[®]

Mouse Periostin/OSF-2 Immunoassay

Catalog Number MOSF20

For the quantitative determination of mouse Periostin concentrations in cell culture supernates, mouse serum, and plasma.

This package insert must be read in its entirety before using this product.

**FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

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INTRODUCTION

Periostin, originally called OSF-2 (osteoblast-specific factor 2), belongs to the FAS1 superfamily (1 - 4). In mammals, the periostin family includes periostin, β Ig-H3, and stabilins 1 and 2 (1 - 5). Periostin is a secreted ~90 kDa monomer or 170 kDa homodimer which is unlikely to be significantly glycosylated. It is primarily expressed during differentiation of mesenchymal cells, in collagen and fibroblast-rich connective tissues and intramembranous bone, and in areas that are under mechanical stress, such as the periosteum, periodontal ligament, and atrioventricular valves (2, 5 - 14). Synthesis by adult fibroblasts, osteoblasts, vascular smooth muscle, and epicardium-derived cells is upregulated after injury (3, 7, 9, 10, 13 - 17). Full-length mature periostin is an 815 amino acid (aa) protein with an N-terminal cysteine-rich EMI domain and four 130 aa fasciculin type 1 (FAS1) domains (18, 19). The FAS1 domains contain gamma-carboxylated glutamate residues, making periostin a vitamin K-dependent protein (20). Mature mouse periostin shares 98%, 92%, and 91% aa sequence identity with rat, dog, and human periostin, respectively. Insertions or deletions at splice sites C-terminal to the FAS1 repeats produce mature molecules of 760 - 788 amino acids and 87 - 93 kDa (1, 7, 21, 22). The splice forms appear to be active and are temporally regulated during heart, bone, tooth, and nerve development, as well as tumorigenesis (21 - 24).

Periostin expression is enhanced by TGF- β , BMP-2, IL-4, and IL-13 but inhibited by EGF and FGF basic (1, 7, 15, 21, 25). It serves as an adhesion molecule by interacting with integrins such as α v β 3, α v β 5, and α 6 β 4 and activates α v, β 1, β 3, and β 5 integrins in cardiomyocytes (10, 13, 26, 27). Periostin induces expression of VEGF R2/KDR/Fik-1 by binding to endothelial cell α v β 3, thus stimulating angiogenesis (27). It may be either upregulated or downregulated in tumor cells and upregulated in surrounding stroma (3, 8, 9, 23, 26, 27). As a mesenchymal cell marker, it indicates epithelial to mesenchymal transition, which often occurs during transformation (3, 26, 27). It can promote encapsulation of tumors with collagen-rich fibers, which negatively regulates tumor growth (28). Elevated plasma periostin has been associated with some, but not all, breast, thymoma, non-small cell lung, and pancreatic ductal carcinomas, especially during bone metastasis (4). Elevation in ascites fluid is found in some ovarian cancers (10).

Periostin binds extracellular matrix proteins including collagens, fibronectin, and tenascin-C and is thought to promote collagen I fibril formation and strengthening (4, 11, 16, 25). It participates in tissue remodeling in a variety of settings, such as wound healing, fibrosis in response to asthma in the lung or myocardial infarction in the heart, or in fibrous dysplasia bone lesions (13, 17, 25). It strengthens the periodontal ligament under the mechanical stress of chewing (12, 29). Periostin deletion in mouse is associated with malformation of the periodontal ligament and cardiac atrioventricular valves (6, 12, 16). Myocardial infarction in periostin-deficient mice initially causes more rupture, but surviving animals show improved recovery due to a decrease in fibrosis as compared to wild-type mice (17, 29, 30).

The Quantikine Mouse Periostin/OSF-2 immunoassay is a 4.5 hour solid-phase ELISA designed to measure Periostin in cell culture supernates, serum, and plasma. It contains Sf 21-expressed recombinant mouse Periostin and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant mouse Periostin. Results obtained using natural mouse Periostin showed dose response curves that were parallel to the standard curves obtained using the Quantikine mouse kit standards. These results indicate that the Quantikine Mouse Periostin/OSF-2 immunoassay can be used to determine relative mass values for natural mouse Periostin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse Periostin has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any mouse Periostin present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse Periostin is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of mouse Periostin bound in the initial step. The sample values are then read off the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with the Calibrator Diluent and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins and other factors present in biological samples. Until all factors have been tested, however, the possibility of interference cannot be excluded.

PRECAUTION

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face and clothing protection when using this material.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- For best results, pipette reagents and samples into the center of each well.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

MATERIALS PROVIDED

Mouse Periostin/OSF-2 Microplate (Part 893714) - One 96 well polystyrene microplate (12 strips of 8 wells) coated with a rat monoclonal antibody specific for mouse Periostin.

Mouse Periostin/OSF-2 Conjugate (Part 893715) - 12 mL of a polyclonal antibody specific for mouse Periostin conjugated to horseradish peroxidase with preservatives.

Mouse Periostin/OSF-2 Standard (Part 893716) - 2 vials (20 ng/vial) of recombinant mouse Periostin in a buffered protein base with preservatives; lyophilized.

Mouse Periostin/OSF-2 Control (Part 893717) - 2 vials of recombinant mouse Periostin in a buffered protein base with preservatives; lyophilized. The concentration range of mouse Periostin after reconstitution is shown on the vial label. The assay value of the Control should be within the range specified on the label.

Calibrator Diluent RD5-26 Concentrate (Part 895525) - 21 mL of a buffered protein solution with preservatives.

Assay Diluent RD1-21 (Part 895215) - 12 mL of a buffered protein solution with preservatives.

Wash Buffer Concentrate (Part 895024) - 50 mL of a 25-fold concentrated solution of buffered surfactant with preservative.

Color Reagent A (Part 895000) - 12 mL of stabilized hydrogen peroxide.

Color Reagent B (Part 895001) - 12 mL of stabilized chromogen (tetramethylbenzidine).

Stop Solution (Part 895174) - 23 mL of diluted hydrochloric acid.

Plate Covers (Part 640197) - 4 adhesive strips.

STORAGE

Unopened Kit	Store at 2 - 8° C. Do not use past kit expiration date.	
Opened/ Reconstituted Reagents	Mouse Periostin/OSF-2 Conjugate	May be stored for up to 1 month at 2 - 8° C.*
	Diluted Wash Buffer	
	Stop Solution	
	Calibrator Diluent RD5-26 (1X)	
	Assay Diluent RD1-21	
	Unmixed Color Reagent A	
	Unmixed Color Reagent B	Discard after use. Use a new standard and control for each assay.
	Mouse Periostin/OSF-2 Standard (10 ng/mL)	
	Mouse Periostin/OSF-2 Control	
	Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.*

*Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 1000 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- **Polypropylene tubes.**

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at $\leq -20^{\circ}$ C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature or overnight at $2 - 8^{\circ}$ C before centrifuging. Centrifuge for 20 minutes at $2000 \times g$. Remove serum and assay immediately or aliquot and store samples at $\leq -20^{\circ}$ C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes at $2000 \times g$ within 30 minutes of collection. Assay immediately or aliquot and store samples at $\leq -20^{\circ}$ C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

SAMPLE PREPARATION

Serum and plasma samples require a 500-fold dilution. A suggested 500-fold dilution is 10 μ L of sample + 90 μ L of Calibrator Diluent RD5-26 (1X) followed by 10 μ L of the diluted sample + 490 μ L of Calibrator Diluent RD5-26 (1X).

Cell culture supernate samples require a 2-fold dilution. A suggested 2-fold dilution is 70 μ L of sample + 70 μ L of Calibrator Diluent RD5-26 (1X).

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse Periostin/OSF-2 Control - Reconstitute the Control with 1.0 mL deionized or distilled water. Assay the Control undiluted.

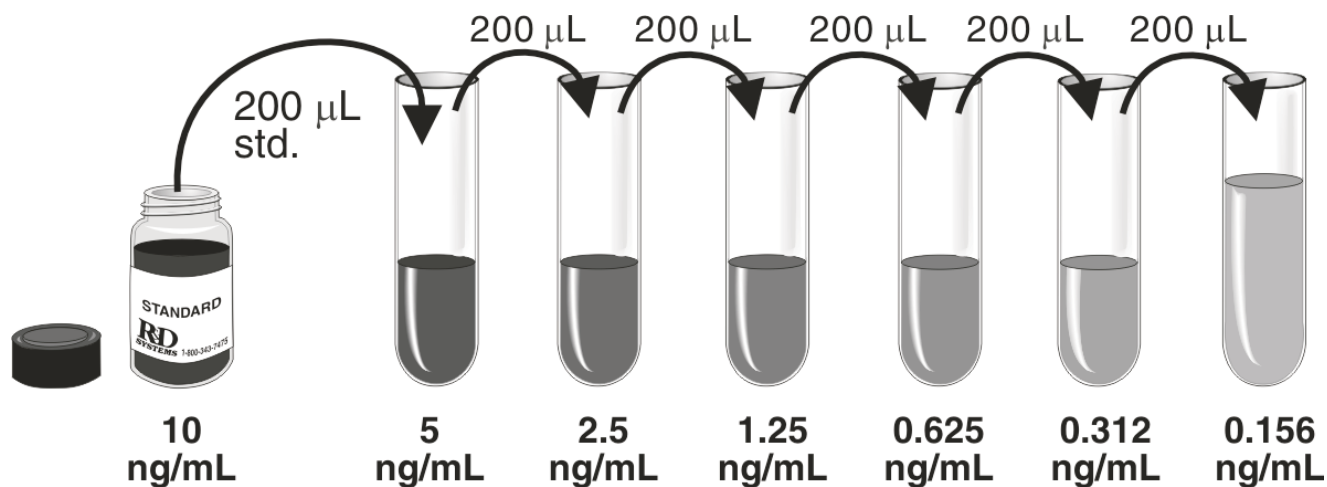
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. To prepare enough Wash Buffer for one plate, add 25 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 625 mL of Wash Buffer.

Calibrator Diluent RD5-26 (1X) - Dilute 20 mL of Calibrator Diluent RD5-26 Concentrate into 60 mL of deionized or distilled water to prepare 80 mL of Calibrator Diluent RD5-26 (1X).

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 μ L of the resultant mixture is required per well.

Mouse Periostin/OSF-2 Standard - Reconstitute the Mouse Periostin/OSF-2 Standard with 2.0 mL of Calibrator Diluent RD5-26 (1X). Do not substitute other diluents. This reconstitution produces a stock solution of 10 ng/mL. Allow the stock solution to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Add 200 μ L of Calibrator Diluent RD5-26 (1X) to each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube gently but thoroughly before the next transfer. The undiluted Mouse Periostin Standard serves as the high standard (10 ng/mL). Calibrator Diluent RD5-26 (1X) serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, control, and standards be assayed in duplicate.

1. Prepare all reagents, standard dilutions, control, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal.
3. Add 50 μ L of Assay Diluent RD1-21 to each well.
4. Add 50 μ L of Standard, Control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 μ L) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 μ L of Mouse Periostin/OSF-2 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 μ L of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 100 μ L of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples require dilution. See the Sample Preparation section.

PROCEDURE SUMMARY AND CHECKLIST

1. Bring all reagents to room temperature.
 Prepare reagents and samples as instructed.
 Return unused components to storage temperature as indicated in the instructions.
2. Add 50 μL Assay Diluent RD1-21 to each well.
3. Add 50 μL Standard, Control, or sample* to each well.
 Cover the plate and incubate for 2 hours at room temperature on the shaker.
4. Aspirate and wash each well five times.
5. Add 100 μL Conjugate to each well.
 Cover the plate and incubate for 2 hours at room temperature on the shaker.
6. Aspirate and wash each well five times.
7. Add 100 μL Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
8. Add 100 μL Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Read Optical Density at 450 nm (correction wavelength set at 540 nm or 570 nm).

*Samples require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

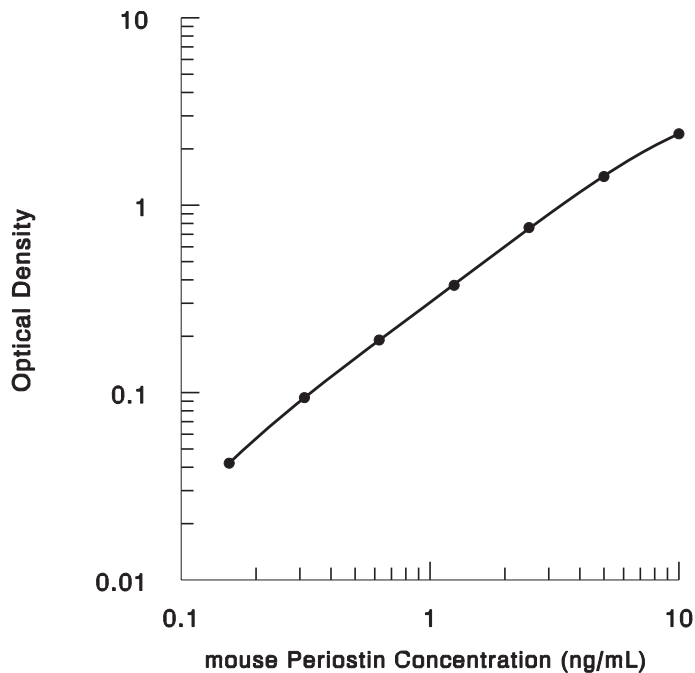
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse Periostin concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

Since samples have been diluted prior to assay, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



ng/mL	O.D.	Average	Corrected
0	0.020 0.020 0.061	0.020	—
0.156	0.062 0.108	0.062	0.042
0.312	0.121 0.211	0.114	0.094
0.625	0.211 0.378	0.211	0.191
1.25	0.410 0.757	0.394	0.374
2.5	0.803 1.423	0.780	0.760
5	1.464 2.410	1.444	1.424
10	2.444	2.427	2.407

PRECISION

Intra-assay Precision (Precision within an assay)

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples of known concentration were tested in forty assays to assess inter-assay precision.

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (ng/mL)	0.600	0.833	4.69	0.629	0.867	4.84
Standard deviation	0.021	0.035	0.117	0.056	0.067	0.260
CV (%)	3.5	4.2	2.5	9.0	7.7	5.4

RECOVERY

The recovery of mouse Periostin spiked to three levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture samples (n=6)	95	81 - 117%

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of mouse Periostin in each matrix were diluted with Calibrator Diluent and assayed.

		Cell culture supernates* (n=6)	Serum* (n=6)	Heparin plasma* (n=6)	EDTA plasma* (n=6)
1:2	Average % of Expected	100	97	100	99
	Range (%)	97 - 103	91 - 104	96 - 106	96 - 103
1:4	Average % of Expected	100	94	100	97
	Range (%)	91 - 111	89 - 101	96 - 106	94 - 103
1:8	Average % of Expected	102	93	102	96
	Range (%)	90 - 116	88 - 99	95 - 111	91 - 102
1:16	Average % of Expected	111	91	99	92
	Range (%)	109 - 112	85 - 99	92 - 108	87 - 99

*Samples were diluted prior to assay as described in the Sample Preparation section.

SENSITIVITY

Sixty-eight assays were evaluated and the minimum detectable dose (MDD) of mouse Periostin ranged from 0.002 - 0.065 ng/mL. The mean MDD was 0.012 ng/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified *Sf 21*-expressed recombinant mouse Periostin produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse Periostin in this assay.

	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Mouse serum* (n=20)	2184	1718 - 2659	288
Mouse heparin plasma* (n=20)	1780	730 - 2714	444
Mouse EDTA plasma* (n=20)	1920	1133 - 2572	324

*Samples were diluted prior to assay as described in the Sample Preparation section.

Cell Culture Supernates -

Mouse bone marrow mast cells (BMMC) were collected from the hind femurs of three 7 - 8 week old NSA mice. The cells were then cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 50 μ M 2-mercaptoethanol, 25 ng/mL recombinant mouse SCF, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin sulfate. The cells were seeded into 100 mL of media and incubated for 7 days. On day 7, all non-attached cells were collected by centrifugation, resuspended in 100 mL of fresh media, and incubated for an additional 7 days. An aliquot of the cell culture supernate was removed, assayed for levels of natural Periostin, and measured 2.34 ng/mL.

Hearts from three mice were chopped into 1 - 2 mm pieces. The cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 50 μ M 2-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin sulfate. The cells were seeded into 100 mL of media for 3 days. An aliquot of the cell culture supernate was removed, assayed for levels of natural Periostin, and measured 2.55 ng/mL.

Lungs from two mice were separated into individual cells using a dounce homogenizer. The cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 50 μ M 2-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin sulfate. The cells were seeded into 100 mL of media for 22 hours. An aliquot of the cell culture supernate was removed, assayed for levels of natural Periostin, and measured 2.41 ng/mL.

Mouse Embryonic Fibroblast (3T3-L1) -

Day 0: Cells were seeded at 2×10^6 cells/mL into 50 mL of growth media (DMEM with glucose, 10% fetal bovine serum, 100 U/mL penicillin, and 100 μ g/mL streptomycin sulfate) in each of two T-175 flasks and grown to confluence.

Day 3: The cell culture supernates were collected from both flasks. 50 mL of fresh growth media was added to one flask (Flask 1) and 50 mL of differentiation media (growth media plus 1 μ g/mL bovine insulin, 0.5 mM MIX, and 1 μ M DEX) was added to the other flask (Flask 2). Both flasks were incubated for 4 days.

Day 7: The cell culture supernates were collected from both flasks. 50 mL of fresh growth media was added to one flask (Flask 1) and 50 mL of growth media with 1 μ g/mL bovine insulin was added to the other flask (Flask 2). Both flasks were incubated for 4 days.

Day 11: The cell culture supernates were collected from both flasks and assayed for levels of natural mouse Periostin.

	Day 3 (ng/mL)	Day 7 (ng/mL)	Day 11 (ng/mL)
Flask 1, Undifferentiated	2.16	27.2	5.83
Flask 2, Differentiated	2.04	382	278

SPECIFICITY

This assay recognizes both recombinant and natural mouse Periostin. The factors listed below were prepared at 100 ng/mL in Calibrator Diluent RD5-26 (1X) and assayed for cross-reactivity. Preparations of the following factors in a mid-range mouse Periostin control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:
 β IG-H3

Recombinant human:
 β IG-H3
Integrin β 5
Periostin

Rat and canine Periostin were not detectable in serum samples in this assay.

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PLATE LAYOUT

Use this plate layout as a record of standards and samples assayed.

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11								
12								
	A	B	C	D	E	F	G	H

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