BioVision
BioVision Incorporated

Periostin/OSF-2 (mouse) ELISA Kit

rev.11/14

(Catalog # K4762-100, 100 assays; Store at 4^{0}C)

I. Introduction:

BioVision's Periostin (mouse) ELISA kit is to be used for the in vitro 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. Detection limit: 10 pg/ml. Note: The Limit of detection was measured by adding three standard deviations to the mean value of 50 zero standard. Assay Range: 31.25 pg/ml – 2000 pg/ml. Linearity: Different samples containing mouse periostin were diluted several fold (1/4000 to 1/8000) and the measured recoveries ranged from 95% to 105%. Mouse periostin levels range in plasma or serum from 1 to >10 µg /ml. Recovery: When samples (serum or plasma) are spiked with known concentrations of mouse periostin, the recovery averages 96% (range from 89% to 115%).

II. Application:

Quantitative ELISA.

III. Specificity:

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

IV. Sample Type:

- Serum & plasma
- Cell culture supernatants

V. Kit Contents:

Components	K4762-100	Part No.
Plate coated with periostin Antibody	6 X 16-well strips	K4762-100-1
Mouse periostin Standard	5 µg	K4762-100-2
Detection Antibody	20 µl	K4762-100-3
HRP Labeled Streptavidin (STREP-HRP)	2 µg	K4762-100-4
10X Wash Buffer	2 X 30 ml	K4762-100-5
10X ELISA Buffer	2 X 30 ml	K4762-100-6
TMB	12 ml	K4762-100-7
Stop Solution	12 ml	K4762-100-8
Plate Covers	2	K4762-100-9
Silica Gel Minibags	2	K4762-100-10

VI. User Supplied Reagents and Equipment:

- Microplate reader at 450 nm, with the correction wavelength set at 540 nm or 570 nm
- 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

VII. Storage and Handling:

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.

VIII. Reagent Preparation:

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

- **Wash Buffer 10X:** Dilute 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 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!
- **Mouse periostin Standard (STD)** has to be reconstituted with 100 µl 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 min. at 37°C. Mix well prior to making dilutions.

Start with the dilution of the concentrate (STD):

To obtain	Add	Into
500 ng/ml	10µl of periostin (STD)(50 µg/ml)	990 µl of ELISA Buffer 1X
20 ng/ml	20µl of periostin (STD)(500 ng/ml)	480 µl of ELISA Buffer 1X

Dilute further for the standard curve:

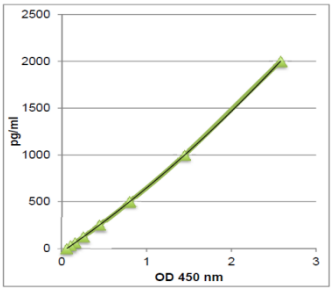
To obtain	Add	Into
2000 pg/ml	100 µl of periostin (20 ng/ml)	900 µl of ELISA Buffer 1X
1000 pg/ml	300 µl of periostin (2000 pg/ml)	300 µl of ELISA Buffer 1X
500 pg/ml	300 µl of periostin (1000 pg/ml)	300 µl of ELISA Buffer 1X
250 pg/ml	300 µl of periostin (500 pg/ml)	300 µl of ELISA Buffer 1X
125 pg/ml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

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.

- **Sample collection, storage and dilution:** Cell Culture Supernatants, serum and plasma: Dilute in 1X ELISA Buffer. Starting dilutions of 1/4000 to 1/8000 are recommended.

IX. Assay Protocol:

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 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 reagent preparation).
3. Cover the plate with plastic film and incubate for 2 hrs at room temperature.
4. Aspirate the coated wells and add 300 µl of 1X Wash Buffer 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 reagent preparation).
6. Cover the plate with plastic film and incubate for 1 hr at room temperature.
7. Aspirate the coated wells and add 300 µl of 1X Wash Buffer 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 reagent preparation).
9. Cover the plate with plastic film and incubate for 30 min. at room temperature.
10. Aspirate the coated wells and add 300 µl of 1X Wash Buffer 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 room temperature in the dark for 10-20 min. 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.
15. **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 axis. 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 mouse periostin in the sample.



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

Figure 1: Standard Curve:

Samples	Means (µg/ml)	SD	CV (%)	n
A1	1.991	0.043	2.14	8
A2	1.017	0.017	1.70	8
A3	2.024	0.072	3.55	8
A4	3.048	0.051	1.67	8

Table 1: Intra-assay precision: Four samples of known con. Of mouse periostin were assayed in replicates 8 times to test precision within an assay.

Samples	Means (µg/ml)	SD	CV (%)	n
B1	1.36	0.096	7.05	3
B2	1.97	0.18	8.98	3
B3	1.36	0.09	6.63	3
B4	1.81	0.14	7.67	3

Table 2: Inter-assay precision: Four samples of known con. of mouse periostin were assayed in 3 separate assays to test precision between assays.

X. RELATED PRODUCTS:

Periostin/OSF-2, human recombinant (4204)

Periostin/OSF-2 (human) ELISA Kit (K4760)

FOR RESEARCH USE ONLY! Not to be used on humans.