# Periostin (mouse) Matched Pair Detection Set

*[*Osteoblast-specific factor 2 (mouse) *Matched Pair Detection Set]*

*Cat. N° AG-46B-0002-KI01* *For Research Use Only* *24-February-2015*

## 1. Intended Use

The Periostin (mouse) Matched Pair Detection Set contains key reagents required to develop sandwich ELISA to measure mouse Periostin. The assay procedure has been optimized for cell culture supernatant and serum.

## 2. Materials Provided

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|---|---|---|---|---|
| • AG-46B-0002/STD | Standard | 1 vial | (5 µg) (*lyophilized*) | (STD) |
| • AG-46B-0002/COAT | Coating Antibody | 1 vial | (120 µl) | (COAT) |
| • AG-46B-0002/DET | Detection Antibody | 1 vial | (20 µl) | (DET) |
| • AG-46B-0002/STREP | Streptavidin-HRP | 1 vial | (10 µg) (*lyophilized*) | (STREP) |

## 3. Materials Required

- PBS: 137mM NaCl, 2.7mM KCl, 8.1mM $Na_2HPO_4$, 1.5mM $KH_2PO_4$, pH 7.2, 0.2µM filtered
- Wash Buffer: 0.1% Tween® 20 in PBS
- Blocking Buffer: 2% BSA (ELISA grade) in PBS, 0.2µM filtered.
- ELISA Buffer: 0.2% BSA (ELISA grade) and 0.05% Tween® 20 in PBS
- TMB: Tetramethylbenzidine
- Plates: Nunc-Immuno MaxiSorp, N°467466
- Stop Solution: 2M sulphuric acid ($H_2SO_4$)
- Distilled or deionized water

## 4. Product Specifications

- Number of Assays: Contains sufficient materials to run ELISAs on 5 x 96-well plates
- Range: 31 pg/ml to 2000 pg/ml
- Specificity: Recognizes mouse Periostin. Does not detect human Periostin.
- Stability: Stable at least 1 year after receipt when stored at -20°C.

## 5. General ELISA Protocol

### a) Reagent Preparation

- Dilute the desired amount of Coating Antibody (COAT) (1 mg/ml) (mouse monoclonal antibody) to 2 µg/ml in PBS without carrier protein and use it fresh.

- Dilute the desired amount of Detection Antibody (DET) (1 mg/ml) (biotinylated mouse monoclonal antibody) to 0.1 µg/ml in ELISA Buffer and use it fresh.

- Reconstitute the Streptavidin-HRP (STREP) with 100 µl ELISA Buffer. After reconstitution, prepare aliquots and store the reconstituted STREP at -20°C. Avoid freeze/thaw cycles. Dilute the reconstituted Streptavidin-HRP to the working concentration by adding 10 µl in 10 ml of ELISA Buffer (1:1000).

- Reconstitute the Standard Protein (STD) (recombinant mouse Periostin) with 100 µl ELISA Buffer to obtain a concentration of 50 µg/ml. After reconstitution, prepare aliquots and store the reconstituted standard at -20°C. Avoid freeze/ thaw cycles. A standard curve using 2-fold serial dilutions in ELISA Buffer is recommended. Suggested standard points are 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.25 pg/ml and 0 pg/ml.

- Cell culture supernatants and serum are diluted in ELISA buffer. For serum, dilutions of >1/4000 are recommended as a start.

*Note that diluted reagents described in this section are not stable and cannot be stored.*

### b) Plate Preparation

1. Coat the wells by adding 100 µl/well of diluted Coating Antibody (2 µg/ml) (see section Reagent Preparation) to a 96-well ELISA microplate (Nunc MaxiSorp™ flat-bottom 96 well plate is suggested). Cover the plate with plastic film and leave overnight (O/N) at 4°C.

2. Aspirate the coated wells. Remove any remaining liquid by inverting the plate and blotting it against clean absorbent paper.

3. Block plates by adding 200 µl of Blocking Buffer for 2 h at room temperature (RT).

4. Aspirate the coated wells and add 300 µl of Wash Buffer using a multichannel pipette or autowasher. Repeat the process for a total of five washes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by inverting the plate and blotting it against clean absorbent paper.

### c) Assay Procedure

1. Add a total of 100 µl/well of cell culture supernatant or serum (diluted in ELISA Buffer) or standard protein (see section Reagent Preparation) to the plate.
2. Cover the plate with plastic film and incubate for 2 h at RT.
3. Repeat the aspiration/wash as in step 4 of Plate preparation.
4. Add 100 µl/well of the diluted Detection Antibody (0.1 µg/ml) (see section Reagent Preparation).
5. Cover the plate with plastic film and incubate for one hour at RT.
6. Repeat the aspiration/wash as in step 4 of Plate preparation.
7. Add 100 µl to each well of the diluted Streptavidin-HRP (see section Reagent Preparation).
8. Cover the plate with plastic film and incubate for 30 min at RT.
9. Repeat the aspiration/wash as in step 4 of Plate preparation.
10. Substrate development is conducted by addition of 100 µl to each well of ready-to-use TMB for 10-30 min at RT.
11. Stop the reaction by adding 50 µl of Stop Solution (2M $H_2SO_4$). Tap the plate gently to ensure thorough mixing.
12. Measure the OD at 450 nm in an ELISA reader.
13. Measure absorbance at 550 nm and subtract these values from those obtained at 450 nm to correct for optical imperfections in the microplate. If absorbance at 550 nm is not possible, measure the absorbance at 450 nm only.
    **Note:** When the 550 nm measurement is omitted, absorbance values will be higher.

## 6. Technical Hints

- Once reagents have been added to the plate, DO NOT let the plate dry at any time during the assay.
- It is recommended that all standards and samples be assayed in duplicate.
- Avoid microbial contamination of reagents and equipment. Automated plate washers can become contaminated thereby causing assay variability. Buffers containing a large quantity of proteins should be made under sterile conditions and stored at 2-8°C or be prepared fresh daily.
- Vigorous plate washing is essential.
- Avoid exposing reagents to excessive heat or light during storage and incubation.
- Wear gloves while performing the assay to avoid contact with samples and reagents. Please follow proper disposal procedure.
- The Stop Solution (STOP) consists of sulfuric acid. Although diluted, the Stop Solution should be handled with gloves, eye protection and protective clothing.

## 7. Calculation of Results

- Average the duplicate readings for each standard, control and sample and subtract the average blank value.

- Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the vertical (Y) axis vs. the corresponding mouse Periostin concentration (ng/ml) on the horizontal axis (see 8. Expected Standard Curve). Calculate results using graph paper or curve-fitting statistical software. The amount of mouse Periostin in each sample is determined by interpolating for the absorbance value (Y axis) using the standard curve.

- If the test samples were diluted, multiply the interpolated values by the dilution factor to calculate ng/ml of mouse Periostin in the samples.

## 8. Expected Standard Curve

The following curve is obtained using the different concentrations of standard as described in this protocol:

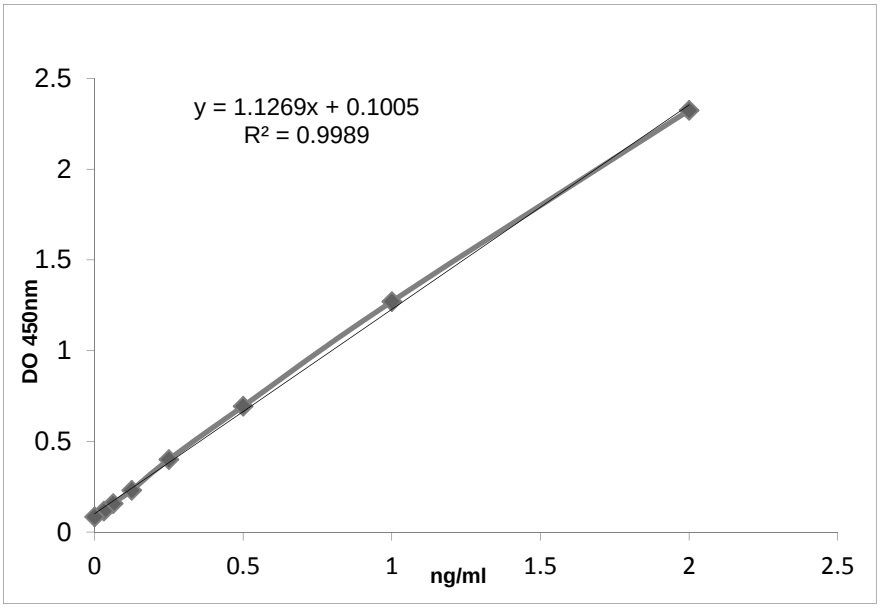


Figure: Standard Curve

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