ab193727 – Periostin Mouse ELISA Kit

Instructions for Use

For the quantitative measurement of mouse Periostin in serum, plasma and cell culture supernatants.

This product is for research use only and is not intended for diagnostic use.

Version 1 Last Updated15 January 2019

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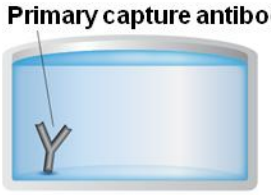
1. BACKGROUND

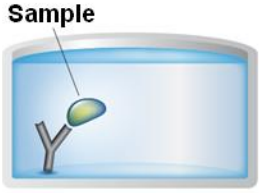
Abcam's Periostin Mouse ELISA Kit (ab193727) is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of mouse Periostin in serum, plasma (Plasma sample should be collected using EDTA or heparin as an anticoagulant. Citrate is not recommended.) and cell culture supernatants.

This assay employs an antibody specific for mouse Periostin coated on a 96-well plate. Standards and samples are pipetted into the wells and the immobilized antibody captures Periostin present in the samples. The wells are washed and biotinylated anti-mouse Periostin antibody is added. After washing away any unbound biotinylated antibody, an HRP-conjugated streptavidin is pipetted to the wells. After incubation, the wells are again washed, followed by the addition of a TMB substrate solution to the wells. Color will develop in proportion to the amount of Periostin bound in each well. Addition of the Stop Solution will change the color from blue to yellow, and the intensity of the color is measured at 450 nm.

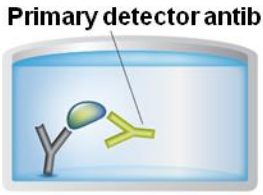
2. ASSAY SUMMARY



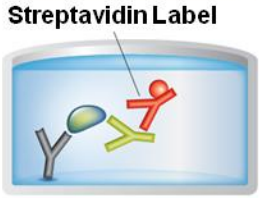
Prepare all reagents, samples and standards as instructed.



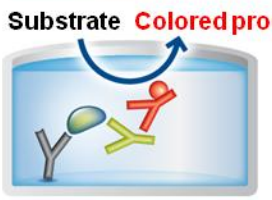
Add standard or sample to each well used. Incubate at room temperature.



Add prepared biotinylated antibody to each well. Incubate at room temperature.



Add prepared streptavidin solution. Incubate at room temperature.



Add TMB One-Step Development Solution to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 9 & 10.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Pre-coated mouse Periostin microplate (12 strips x 8 wells)	96 wells	-20ºC
20X Wash Buffer Concentrate	25 mL	-20ºC
Mouse Periostin Standards	2 vials	-20ºC
Assay Diluent A	30 mL	-20ºC
5X Assay Diluent B	15 mL	-20ºC
Detection Antibody Periostin (biotinylated anti-mouse Periostin)	2 vials	-20ºC
300X HRP-Streptavidin concentrate	200 µL	-20ºC
TMB One-Step Substrate Reagent	12 mL	-20ºC
Stop Solution: sulfuric acid	8 mL	-20ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2 µL to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- Tubes to prepare standard or sample dilutions.

7. LIMITATIONS

- Do not mix or substitute reagents or materials from other kit lots or vendors.

8. TECHNICAL HINTS

- Samples which generate values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Completely aspirate all solutions and buffers during wash steps. When preparing your standards, it is critical to briefly centrifuge the vial first. The powder may adhere to the cap and not be included in the standard solution resulting in an incorrect concentration. Be

sure to dissolve the powder thoroughly when reconstituting. After adding Assay Diluent to the vial, we recommend inverting the tube a few times, then flick the tube a few times, and centrifuge briefly; repeat this procedure 3-4 times. This is an effective technique for thorough mixing of the standard without using excessive mechanical force.

- Do not vortex the standard during reconstitution, as this will destabilize the protein.
- Once your standard has been reconstituted, it should be used right away or else frozen for later use.
- Keep the standard dilutions on ice during preparation, but the ELISA procedure should be done at room temperature.
- Be sure to discard the working standard dilutions after use – they do not store well.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Scientific Support staff with any questions.**

9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18-25°C) prior to use.

9.1 **1X Assay Diluent B**

Dilute 5X Assay Diluent B 5-fold with deionized or distilled water before use.

9.2 **1X Wash Buffer**

If the 20X Wash Concentrate contains visible crystals, equilibrate to room temperature and mix gently until dissolved. Dilute 20 mL of 20X Wash Buffer Concentrate into 380 mL of deionized or distilled water to yield 400 mL of 1X Wash Buffer.

9.3 **Detection Antibody Periostin (biotinylated anti-mouse Periostin)**

Briefly centrifuge the Detection Antibody vial before use. Add 100 µL of 1X Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent B and used in Assay Procedure.

9.4 **Assay Diluent A**

Ready to use.

9.5 **1X HRP-Streptavidin Solution**

Briefly centrifuge the 300X HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use. The 300X HRP-Streptavidin concentrate should be diluted 300-fold with 1X Assay Diluent B.

For example: Briefly centrifuge the vial and pipette up and down to mix gently. Add 40 µL of HRP-Streptavidin concentrate into a tube with 12 mL 1X Assay Diluent B to prepare a 1X HRP-Streptavidin solution (do not store the diluted solution for next day use). Mix well.

10. STANDARD PREPARATIONS

- **Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.**
- **Standard (recombinant protein) should be stored at -20°C or -80°C (recommended at -80°C) after reconstitution.**

10.1 Briefly centrifuge the vial of mouse Periostin Standard and then add 400 µL Assay Diluent A (for serum/plasma samples) or 1X Assay Diluent B (for cell culture supernatants) into the mouse Periostin Standard vial to prepare a 100 ng/mL **Stock Standard**. Mix thoroughly but gently.

10.2 Label tubes #1-8.

10.3 Prepare the 16 ng/mL **Standard #1** by adding 96 µL Stock Standard into tube #1 along with 504 µL Assay Diluent A or 1X Assay Diluent B. Mix thoroughly but gently.

10.4 Add 300 µL Assay Diluent A or 1X Assay Diluent B into tubes 2-8.

10.5 Prepare **Standard #2** by adding 200 µL Standard #1 to tube #2. Mix thoroughly but gently.

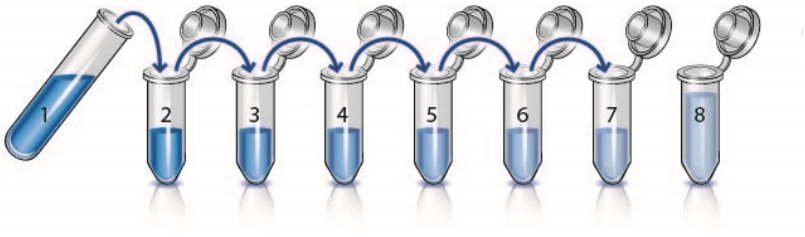
10.6 Prepare **Standard #3** by adding 200 µL from Standard #2 to tube #3. Mix thoroughly but gently.

10.7 Using the table below as a guide, prepare further serial dilutions.

10.8 Standard #8 contains no protein and is the Blank control.

Standard Dilution Preparation Table

Standard #	Sample to Dilute	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	Stock	96	504	100	16
2	Standard #1	200	300	16	6.4
3	Standard #2	200	300	6.4	2.56
4	Standard #3	200	300	2.56	1.024
5	Standard #4	200	300	1.024	0.410
6	Standard #5	200	300	0.410	0.164
7	Standard #6	200	300	0.164	0.066
8 (Blank)	none	-	300	0	0



11. SAMPLE PREPARATION

- If your samples need to be diluted, Assay Diluent A should be used for dilution of serum/plasma samples. 1X Assay Diluent B should be used for dilution of culture supernatants.
- Suggested dilution for normal serum/plasma: 50-500 fold.
- Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For each assay performed, a minimum of 2 wells must be used as blanks, omitting primary antibody from well additions.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay.

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature (18 - 25°C) prior to use.**

  It is recommended to assay all standards, controls and samples in duplicate.

13.1. Add 100 µL of each standard (see Standard Preparations, section) and sample into appropriate wells. Cover plate and incubate for 2.5 hours at room temperature or overnight at 4°C with gentle shaking.

13.2. Discard the solution and wash 4 times with 1X Wash Buffer. Wash by filling each well with 300 µL 1X Wash Buffer using a multi-channel Pipette 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.

13.3. Add 100 µL of the prepared biotinylated mouse Periostin Detection Antibody (see Reagent Preparation section) to each well. Incubate for 1 hour at room temperature with gentle shaking.

13.4. Discard the solution. Repeat the wash as in step 13.2.

13.5. Add 100 µL of prepared 1X HRP-Streptavidin solution (see Reagent Preparation section) to each well. Incubate for 45 minutes at room temperature with gentle shaking

13.6. Discard the solution. Repeat the wash as in step 13.2.

13.7. Add 100 µL of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

13.8. Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

14. CALCULATIONS

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average Blank absorbance value. Plot the standard curve on log-log graph paper, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

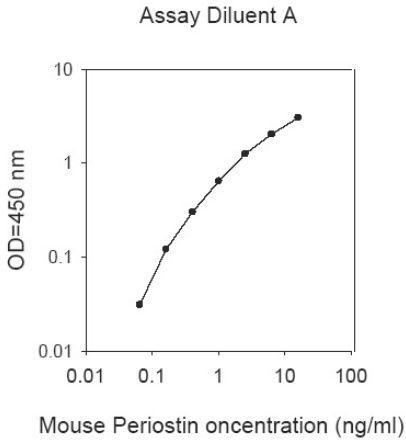


Figure 1. Example of typical mouse Periostin standard curve using Assay Diluent A. The standard curve was prepared as described in Section 10.

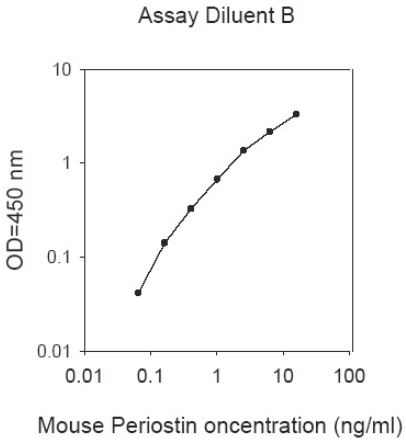


Figure 2. Example of typical mouse Periostin standard curve using Assay Diluent B. The standard curve was prepared as described in Section 10.

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The minimum detectable dose of Periostin is 0.05 ng/mL.

RECOVERY –

Recovery was determined by spiking various levels of Periostin into normal mouse serum, plasma and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	116.6	88-145
Plasma	109.0	84-134
Cell culture media	107.0	91-121

LINEARITY OF DILUTION -

Serum Dilution	Average % Expected Value	Range (%)
1:2	142.5	134-150
1:4	93.66	113-133

Plasma Dilution	Average % Expected Value	Range (%)
1:2	95.89	70-122
1:4	89.16	67-112

Cell Culture Media Dilution	Average % Expected Value	Range (%)
1:2	97.55	89-106
1:4	73.02	67-80

PRECISION –

	Intra-Assay	Inter-Assay
%CV	<10	<12

17. ASSAY SPECIFICITY

The antibodies used within this ELISA kit detect mouse Periostin.

The reaction of these antibodies with other species has not been tested.

Does not cross react with Axl, BLC, CD27 Ligand, CD30, CD30 Ligand, CD40, CXCL16, EGF, E-selectin, Fractalkine, GITR, HGF, ICAM-1, IFN-gamma, IGF-1, IGFBP-2, IGFBP-3, IGFBP-5, IGFBP-6, IL-1 alpha, IL-1 ra, IL-1 beta, IL-2, IL-2 R alpha, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12 p70, IL-17E, IL-17F, IL-20, IL-23, IL-28, I-TAC, MCP-1, MCP-5, M-CSF, MDC, MIG, MIP-1 alpha, MIP-1 gamma, MIP-2, MIP-3 alpha, Osteopontin, Osteoprotegerin, Prolactin, Pro-MMP-9, P-selectin, Resistin, SCF, SDF-1 alpha, TPO, VCAM-1.

Please contact our Scientific Support team for more information.

18. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipette performance.
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation time; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipette performance and ensure correct preparation
High %CV	Inaccurate pipetting	Check pipette performance
High background	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the reconstituted protein at -80°C, all other assay components 4°C. Keep substrate solution protected from light.
	Stop solution	Stop solution should be added to each well before measuring

19. NOTES

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