Nori™ Equine PDGF-AA ELISA Kit-DataSheet

Platelet-derived growth factor subunit A is a protein that in humans is encoded by the *PDGFA* gene (1,2). The protein is a member of the platelet-derived growth factor family. The four members of this family are mitogenic factors for cells of mesenchymal origin and are characterized by a motif of eight cysteines. This gene product can exist either as a homodimer or as a heterodimer with the platelet-derived growth factor beta polypeptide, where the dimers are connected by disulfide bonds. Studies using knockout mice have shown cellular defects in oligodendrocytes, alveolar smooth muscle cells, and Leydig cells in the testis; knockout mice die either as embryos or shortly after birth. Two splice variants have been identified for this gene.

Though it is synthesized, stored and released by platelets upon activation, it is produced by a plethora of cells including smooth muscle cells, activated macrophages, and endothelial cells. PDGFs are mitogenic during early developmental stages, driving the proliferation of undifferentiated mesenchyme and some progenitor populations. During later maturation stages, PDGF signalling has been implicated in tissue remodelling and cellular differentiation, and in inductive events involved in patterning and morphogenesis. In addition to driving mesenchymal proliferation, PDGFs have been shown to direct the migration, differentiation and function of a variety of specialised mesenchymal and migratory cell types, both during development and in the adult animal (3).

Reference

1. Stenman G, Rorsman F, Huebner K, Betsholtz C (Sep 1992). "The human platelet-derived growth factor alpha chain (PDGFA) gene maps to chromosome 7p22". *Cytogenet Cell Genet* **60** (3–4): 206–7.
2. Matsui T, Heidaran M, Miki T, Popescu N, La Rochelle W, Kraus M, Pierce J, Aaronson S (Mar 1989). "Isolation of a novel receptor cDNA establishes the existence of two PDGF receptor genes". *Science* **243** (4892): 800–4.
3. Hoch RV, Soriano P (2003). "Roles of PDGF in animal development". *Development* **130** (20): 4769–4784.

PRINCIPLE OF THE ASSAY

This is a shorter ELISA assay that reduces time to 50% compared to the conventional method, and the entire assay only takes 3 hours. This assay employs the quantitative sandwich enzyme immunoassay technique and uses biotin-streptavidin chemistry to improve the performance and the sensitivity of the assays. An antibody specific for equine PDGF-AA has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any PDGF-AA present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for equine PDGF-AA is added to the wells. Following wash to remove any unbound antibody reagent, a detection reagent is added. After intensive wash a substrate solution is added to the wells and color develops in proportion to the amount of PDGF-AA bound in the initial step. The color development is stopped and the intensity of the color is measured.

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

Storage

Store the standard, detection antibody and the antibody pre-coated plate at 4°C or -20°C, 10 x reagent diluents at -20°C, and store the rest of the kit at 4°C. The kit can be used in 3 months.

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MATERIALS PROVIDED

Description	Quantity	Description	Quantity	Description	Quantity
Antibody Precoated Plate	1	20 x PBS	1	Substrate Solution	1
Detection Antibody	1	20 x Wash Buffer	1	Stop Solution	1
Detection Agent	1	10 x Reagent Diluent	1	DataSheet/Manual	1
Standard	3	Standard Diluent	1	96-well plate sheet	1

Bring all reagents to room temperature before use.

1 x **96-well Plate precoated with Equine PDGF-AA antibody**-Store at 4°C or -20°C upon received.

Equine PDGF-AA Detection Antibody– The lyophilized Detection Antibody should be stored at 4°C or -20°C in a manual defrost freezer for up to 6 months, if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for a 96-well plate. Add 200 µL of 1 x Reagent Diluent to the antibody vial, vortex 20 sec and allow it to sit for 5 min. Take 200 µL of detection antibody to 9.8 mL 1 x Reagent Diluent if the entire 96-well plate is used. If the partial antibody is used store the rest at -20°C until use.

Equine PDGF-AA Standard (3 vials) – The lyophilized equine PDGF-AA Standard has 3 vials. Each vial contains the standard sufficient for generating a standard curve. The non-reconstituted standard can be stored at 4°C or -20°C for up to 3 months if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. Add 500 µL of Standard Diluent to a standard vial to make the high standard concentration of 1200 pg /ml. Vortex 20 sec and allow it to sit for 5 min prior to use. A seven point standard curve is generated using 2-fold serial dilutions in Standard Diluent, vortex 20 sec for each of dilution steps.

Detection Agent (50 µL) – Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for a 96-well plate. If the volume is less than 50 µL, add 1 x Reagent Diluent to a final volume of 50 µL and vortex briefly. Make 1:200 dilutions in Reagent Diluent. If the entire 96-well plate is used, add 50 µL of Detection Agent to 10 mL Reagent Diluent prior to the assay. The rest of undiluted Detection Reagent can be stored at 4°C for up to 6 months. DO NOT FREEZE.

20 x PBS, pH 7.3, 30 mL- Dilute to 1 x PBS with deionized distilled water and mix well prior to use.
20 x Wash Buffer, 20 mL- Dilute to 1 x Wash Buffer with 1 x PBS prior to use.
10 x Reagent Diluent, 3 mL – Stored at -20°C. Prior to use dilute to 1 x Reagent Diluent with 1 x PBS.
Standard Diluent, 10 mL
Substrate Solution, 10 mL.
Stop Solution, 5 mL.

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Assay Procedure

1. Lift the plate cover from the top left corner and cover the wells that are not used. Vortex briefly the samples and the standards prior to the assay. Add 100 µL of sample (such as plasma or serum) or standard to each well, use duplicate wells for each of the standards and samples, cover the 96-well plate and incubate 1 hour at room temperature.
2. Aspirate each well and wash with 1 x Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with 1 x Wash Buffer (300 µL) using a multi-channel pipette, manifold dispenser or auto-washer. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Add 100 µL of the working dilution of the Detection Antibody to each well. Cover the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2.
5. Add 100 µL of the working dilution of Detection Agent to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2.
7. Add 100 µL of Substrate Solution to each well. Incubate for 5 minutes at room temperature. Avoid placing the plate in direct light.
8. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, 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.

Precaution and Technical Notes

1. A standard curve should be generated for each set of samples assayed. Thorough mixing of the standards at each of dilution steps is critical to ensure a normal standard curve.
2. If PDGF-AA exceeds the upper limit of the detection, the sample needs to be diluted with 1 x Wash Buffer. The dilution factor must be used for calculation of the concentration.
3. Detection Agent contains enzyme, DO NOT mass up with Detection Antibody.
4. The Stop Solution is an acid solution, handle with caution.
5. This kit should not be used beyond the expiration date on the label.
6. A thorough and consistent wash technique is essential for proper assay performance. Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by aspiration or by inverting the plate and blotting it against clean paper towels.
7. Use a fresh reagent reservoir and pipette tips for each step.
8. It is recommended that all standards and samples be assayed in duplicate.
9. Avoid microbial contamination of reagents and buffers. This may interfere with the sensitivity of the assay.

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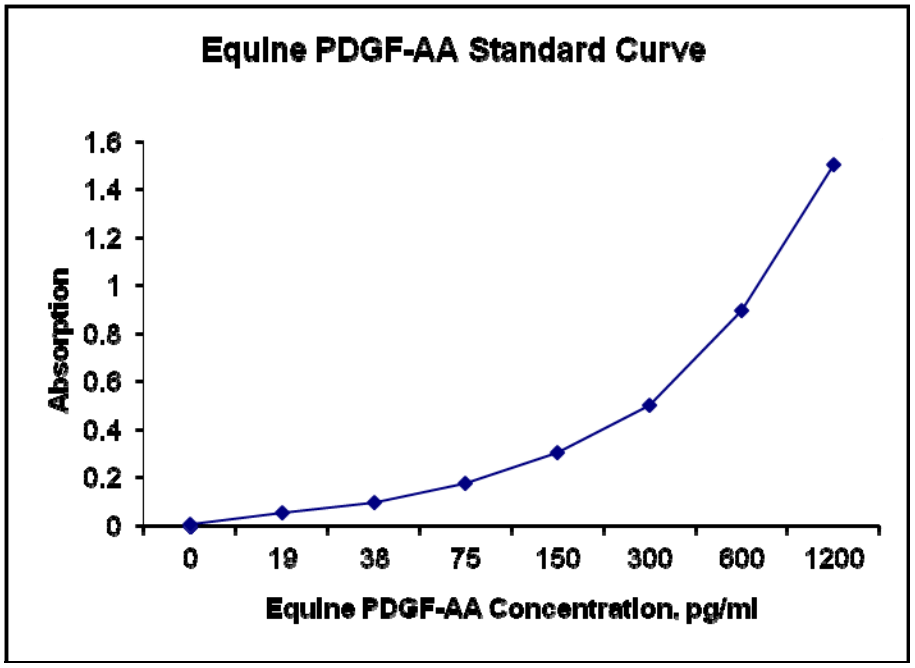
Calculation of Results

Average the duplicate readings for each standard, control, and sample and subtract the average zero (blank) 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 PDGF-AA 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. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

The Standard Curve

The graph below represents typical data generated when using this Equine PDGF-AA ELISA Kit. The standard curve was calculated using a computer generated 4-PL curve-fit. For this case, a Bio-Rad iMark™ Microplate Reader and a Microplate Manager 6 Software were used to generate this curve. The correlation coefficient (r^2) is 0.999-1.000.



Specificity

The following recombinant equine proteins prepared at 10 ng/ml were tested and exhibited no cross-reactivity or interference.
BMP1, BMP2, BMP4, HGF, IL-1β, IL-1RA, IL-2, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-15, IFNγ, MMP-2, MMP-9, sIL-2R, sIL-6R, TGFβ1, TGFβ2, TGFβ3, TLR1, TLR2, TLR3, TNF-α, TNF RI, TNF RII, VEGF.

Calibration

This kit is calibrated against a highly purified CHO cell-expressed recombinant equine PDGF-AA.

Detection Range

1-1200 pg/ml

Assay Sensitivity

0.6 pg/ml

Assay Precision

Intra-Assay %CV: 6; Inter-Assay %CV: 9

For Research Use Only

Related products

20 x Sample Diluent, GERC-103058
20 x PBS, Cat. 103004-20
10 x ELISA Wash Buffer, Cat. 103028
10 x ELISA Reagent Diluent, Cat. GERC-103055
Universal Blocking Buffer, Cat.103005
2 x Recombinant Protein Stabilizer, Cat. GERC-03014-2
5 x Recombinant Protein Stabilizer, Cat. GERC-103014-5
ELISA G-Blue Substrate Solution, Cat. 103021
Equine PDGF-AA Standard
Equine PDGF-AA detection antibody