BioLegend®

Enabling Legendary Discovery™

Human PlGF

ELISA MAX™ *Deluxe Set*

Cat. No. 446004

BioLegend's ELISA MAX™ Deluxe Set contains the components necessary for the accurate quantification of natural and recombinant human PlGF. The set is designed for cost-effective and accurate quantification of human PlGF in cell culture supernatant, serum, urine or other biological fluids. BioLegend's ELISA MAX™ Deluxe Sets are sensitive, accurate, and robust.

It is highly recommended that this instruction sheet be read in its entirety before using this product. Do not use this set beyond the expiration date.

Materials Provided

1. Human PlGF ELISA MAX™ Capture Antibody (200X)
2. Human PlGF ELISA MAX™ Detection Antibody (200X)
3. Human PlGF Standard
4. Avidin-HRP (1000X)
5. Substrate Solution F
6. Coating Buffer B (5X)
7. Assay Diluent A (5X)
8. Matrix Diluent A

Introduction

Placenta growth factor (PlGF) is a member of the PDGF/VEGF family of growth factors. It is expressed in placenta, microvascular, bone marrow, human umbilical vein endothelia, uterine keratinocytes, and natural killer cells. It induces monocyte activation, migration, production of inflammatory cytokines and tumor formation. Also, PlGF plays a key role in maternal vascular function during pregnancy and can be used as a marker for pregnancy associated hypertensive disorder.

Principle of the Test

BioLegend ELISA MAX™ Deluxe Set contains pre-optimized essential reagents and additional buffers and solutions for Sandwich ELISA assay. A human PlGF specific mouse monoclonal antibody is first coated on a 96-well plate. Standards and samples are added to the wells, and PlGF binds to the immobilized capture antibody. Next, a biotinylated mouse monoclonal anti-human PlGF detection antibody is added, producing an antibody-antigen-antibody "sandwich". The Avidin-HRP reagent is subsequently added, followed by TMB Substrate, producing a blue color in proportion to the concentration of PlGF present in wells. Then Stop Solution should be added to wells to terminate the reaction. This step changes the reaction color from blue to yellow. The absorbance in wells should be read at 450nm using a microplate reader.

For research purposes only. Not for use in diagnostic or therapeutic procedures.

Materials to be Provided by the End-User

- Microwell plates: BioLegend Cat. No. 423501 is recommended
- Wash Buffer: BioLegend Cat. No. 421601 is recommended, or PBS + 0.05% Tween-20
- Stop Solution: BioLegend Cat. No. 423001 is recommended, or acid solution, e.g. 2N H_2SO_4
- Plate Sealers: BioLegend Cat. No. 423601 is recommended
- PBS (Phosphate-Buffered Saline): 8.0 g NaCl, 1.16 g Na_2HPO_4, 0.2 g KH_2PO_4, 0.2 g KCl, add deionized water to 1 L; pH to 7.4, 0.2 µm filtered
- Deionized (DI) water
- A microplate reader capable of measuring absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 2 µL to 1 mL
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer

Storage Information

- Store kit components between 2°C and 8°C.
- After reconstitution of the lyophilized standard with 1X Assay Diluent A, aliquot into polypropylene vials and store at -70°C for up to one month. Avoid repeated freeze/thaw cycles.
- Prior to use, bring all components to room temperature (18°C-25°C). Upon assay completion, return all components to appropriate storage conditions.

Health Hazard Warnings

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details (www.biolegend.com/msds).
2. Substrate Solution F is harmful if ingested. Additionally, avoid skin, eye or clothing contact.
3. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling

Cell Culture Supernatant: If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at < -20°C. Avoid repeated freeze/thaw cycles.

Serum: Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at 1,000 X *g*. Remove serum layer and assay immediately or store serum samples at < -20°C. Avoid repeated freeze/thaw cycles. Serum specimens should be clear and non-hemolyzed.

Urine: Collect the first urine of the day (mid-stream) directly into a sterile container. Centrifuge to remove particulate matter. Assay immediately or aliquot and store samples at < -70°C. Avoid repeated freeze-thaw cycles.

Reagent and Sample Preparation

Do not mix reagents from different sets or lots. Reagents and/or antibodies from different manufacturers should not be used with this set. All reagents should be diluted immediately prior to use.

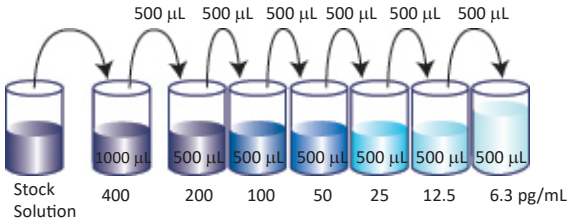
NOTE: Precipitation of 5X Assay Diluent A may be observed when stored long term between 2°C and 8°C. The precipitation does not alter the performance of the Buffer. If heavy precipitation is observed after the dilution to 1X Assay Diluent A, it can be filtered to clarify the solution.

Preparation of 1X Reagent for 1 Plate

Material	Dilute with
2.4 mL of Coating Buffer B (5X)	9.6 mL of Deionized Water
60 µL of Capture Antibody (200X)	12 mL of 1X Coating Buffer B
12 mL of Assay Diluent A (5X)	48 mL of PBS
60 µL of Detection Antibody (200X)	12 mL of 1X Assay Diluent A
12 µL of Avidin-HRP (1,000X)	12 mL of 1X Assay Diluent A

Lyophilized vials are under vacuum pressure. Reconstitute lyophilized standard with 0.2 mL of 1x Assay Diluent A. Allow the reconstituted standard to sit for 15 minutes at room temperature, then mix gently prior to making dilutions.

Prior to use, prepare 1,000 µL of the top standard at a concentration of 400 pg/mL from the stock solution in 1X Assay Diluent A. Perform six two-fold serial dilutions of the 400 pg/mL top standard with 1X Assay Diluent A in separate tubes. After diluting, the human PlGF standard concentrations are 400 pg/mL, 200 pg/mL, 100 pg/mL, 50 pg/mL, 25 pg/mL, 12.5 pg/mL, and 6.3 pg/mL, respectively. 1X Assay Diluent A serves as the zero standard (0 pg/mL).



Samples: In general, samples are analysed without dilution. However, if dilutions are required, use 1X Assay Diluent A as the sample diluent for cell culture supernatant and urine samples, and use Matrix Diluent A as the sample diluent for serum.

Assay Procedure

Do not use sodium azide in any solutions as it inhibits the activity of the horseradish-peroxidase enzyme.

1. One day prior to running the ELISA, dilute Capture Antibody in 1X Coating Buffer B as described in Reagent Preparation. Add 100 µL/well of this Capture Antibody solution to the 96-well plate. Seal plate and incubate overnight between 2°C and 8°C.
2. Bring all reagents to room temperature (RT) prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.
3. Wash plate 4 times with at least 300 µL Wash Buffer per well and blot residual buffer by firmly tapping plate upside down on absorbent paper. **All subsequent washes should be performed similarly.**
4. To block non-specific binding and reduce background, add 200 µL 1X Assay Diluent A per well.
5. Seal plate and incubate at RT for 1 hour with shaking on a plate shaker (e.g. 500 rpm with a 0.3 cm circular orbit). All subsequent incubation with shaking should be performed similarly.
6. While plate is being blocked, prepare the appropriate sample dilutions (if necessary) and standards.
7. Wash plate 4 times with Wash Buffer.

8. For measuring urine samples or cell culture supernatant:
   a) Add 50 µL of 1x Assay Diluent A to each well that will contain either standard dilutions or samples.
   b) Add 50 µL of standard dilutions or samples to the appropriate wells.
   For measuring serum samples:
   a) Add 50 µL of Matrix Diluent A to each well that will contain the standard dilutions. Add 50 µL of 1x Assay Diluent A to each well that will contain samples.
   b) Add 50 µL of standard dilutions to the wells containing Matrix Diluent A. Add 50 µL of serum samples to the wells containing 1x Assay Diluent A.
9. Seal plate, shake for 1 minute and incubate overnight between 2°C and 8°C.
10. Wash plate 4 times with Wash Buffer.
11. Add 100 µL/well of diluted Detection Antibody solution, seal plate and incubate at RT for 2 hours with shaking.
12. Wash plate 4 times with Wash Buffer.
13. Add 100 µL/well of diluted Avidin-HRP solution, seal plate and incubate at RT for 30 minutes with shaking.
14. Wash plate 5 times with Wash Buffer. For this final wash, soak wells in Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
15. Add 100 µL/well Substrate Solution F and incubate **in the dark** for 15 minutes. Positive wells should turn blue in color. It is not necessary to seal the plate during this step.
16. Stop reaction by adding 100 µL/well of Stop Solution. Positive wells should turn from blue to yellow.
17. Read absorbance at 450 nm within 15 minutes. If the reader can read at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

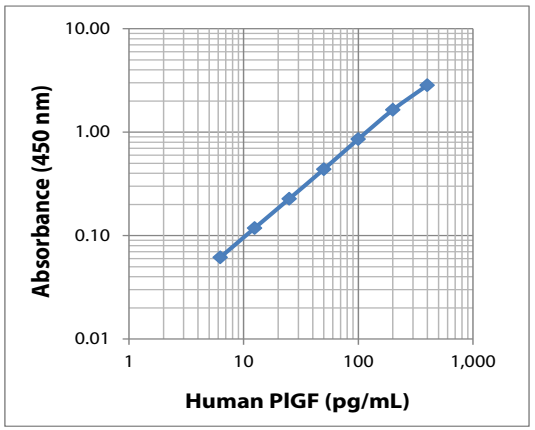
***Optimal substrate incubation time depends on laboratory conditions and the optical linear ranges of ELISA plate readers.**

Calculation of Results

The data is best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If the samples were diluted, multiply by the appropriate dilution factor. If a test sample's absorbance value falls outside the standard curve ranges, that test sample needs to be reanalyzed at a higher or lower dilution as appropriate.

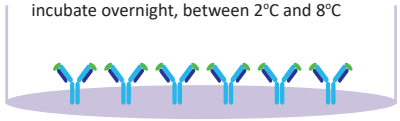
Typical Data

Standard Curve: This standard curve was generated at BioLegend for demonstration purposes only. A standard curve must be run with each assay.

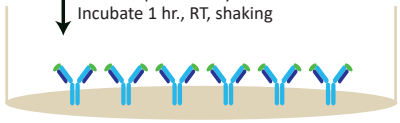


Assay Procedure Summary

1. Coat plate with 100 µL diluted Capture Antibody incubate overnight, between 2°C and 8°C



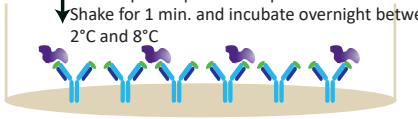
2. Wash 4 times
   Add 200 µL 1X Assay Diluent A
   Incubate 1 hr., RT, shaking



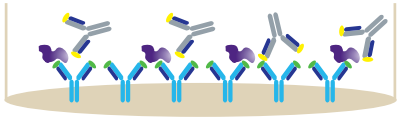
3. **For measuring serum:**
   Wash 4 times
   Add 50 µL Matrix Diluent A to standard wells
   Add 50 µL 1x Assay Diluent A to sample wells

   For measuring urine or cell culture supernatant:
   Wash 4 times
   Add 50 µL 1x Assay Diluent A to standard and sample wells

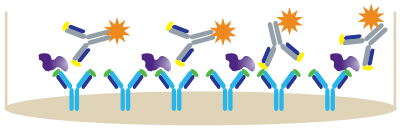
4. Add 50 µL diluted standards to standard wells
   Add 50 µL samples to sample wells
   Shake for 1 min. and incubate overnight between 2°C and 8°C



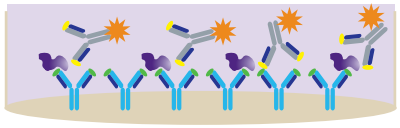
5. Wash 4 times
   Add 100 µL diluted Detection Antibody
   Incubate 2 hr., RT, shaking



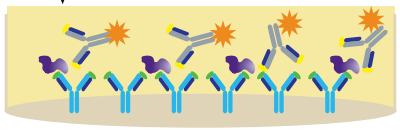
6. Wash 4 times
   Add 100 µL Avidin-HRP
   Incubate 30 min. RT, shaking



7. Wash 5 times
   Add 100 µL Substrate Solution F
   Incubate 15 min. RT, in the dark



8. Add 100 µL Stop Solution



9. Read absorbance at 450nm and 570nm

Performance Characteristics

Sensitivity: The expected minimum detectable concentration of PlGF for this set is 6.3 pg/ml.

Specificity: This set recognizes natural and recombinant PlGF-1. Recombinant PlGF-2 and PlGF-3 show 91.2% and 48.4% reactivity respectively with this assay. Recombinant human VEGF/PlGF heterodimer shows 0.12% cross-reactivity at 50 ng/mL. Recombinant human VEGFR1 starts to interfere at concentrations greater than 2000 pg/mL.

Troubleshooting

High Background:

- Background wells were contaminated.
- Matrix used had endogenous analyte.
- Plate was insufficiently washed.
- TMB Substrate Solution was contaminated.

No signal:

- Incorrect or no antibodies were added.
- Avidin-HRP was not added.
- Substrate solution was not added.
- Wash buffer contains sodium azide.

Low or poor signal for the standard curve:

- Standard was incompletely reconstituted or was stored improperly.
- Reagents were added to wells with incorrect concentrations.
- Plate was incubated with improper temperature, timing or agitation.

Signal too high, standard curves saturated:

- Standard was reconstituted with less volume than required.
- One or more reagent incubation steps were too long.
- Plate was incubated with inappropriate temperature, timing, or agitation.

Sample readings out of range:

- Samples contain no or below detectable levels of analyte.
- Samples contain analyte concentrations greater than highest standard point.

High variations in samples and/or standards:

- Pipetting errors may have occurred.
- Plate washing was inadequate or non-uniform.
- Samples were not homogenous.
- Samples or standard wells were contaminated.

BioLegend, Inc.

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