Human PLGF (Placenta growth factor) ELISA Kit

Enzyme Immunoassay for the quantification of human Placenta Growth Factor (PLGF) in serum

Catalog number: ARG80497

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

Agiogenesis and vascular transformation are important processes in the normal development of the placenta. Abnormal angiogenesis and vascular transformation are considered to be one of the main reasons for preeclamptic pregnancies and intrauterine growth retardation. Placental growth factor PLGF, a member of the VEGF family, is produced mainly by the placenta and is a potent angiogenic factor. The corresponding receptor, the soluble fms-like tyrosine kinase-1 is considered to have angiogenic properties.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for PLGF has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any PLGF present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for PLGF is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of PLGF bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of PLGF in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	1 plate	4°C
Zero Standard	1 vial (1ml) (Ready-to-use)	4°C
Standards 1-5	5 vials (25, 50, 125, 500, 1000 pg/ml)(1ml per vial) (Ready-to-use)	4°C
Control (Low and High)	2 vials (1ml each) (Ready-to-use)	4°C
Biotin-Conjugated Antibody (biotinylated anti-human PLGF antibody)	1 vial (14ml) (Ready-to-use)	4°C
Enzyme Complex	1 vial (14ml) (Ready-to-use)	4°C
Assay Buffer	30ml (Ready-to-use)	4°C
40X Wash buffer	30 ml	4°C
TMB substrate	14 ml	4°C (Protect from light)
STOP solution	14 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 40X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 40X Wash buffer into distilled water to yield 1X Wash buffer.
- Samples: In an initial assay, if a specimen is found to contain more than the highest standard, the specimens can be diluted with Assay buffer and re-assay. For the calculation of concentration this dilution factor has to be taken into account.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 25 µl of standards, controls and samples in duplicate into wells.
3. Add 250 µl Assay Buffer into each well.
4. Incubate for 30 mins at RT without shaking.
5. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add 100 µl Biotin-conjugated antibody into each well.
7. Incubate the plate for 60 minutes at RT.

8. Aspirate each well and wash as step 5.
9. Add 100 µl of Enzyme complex to each well. Incubate for 30 mins at RT.
10. Aspirate each well and wash as step 5.
11. Add 100 µl of TMB Reagent to each well. Incubate for 30 minutes at room temperature in dark.
12. Add 100 µl of Stop Solution to each well. The color of the solution should change from blue to yellow.
13. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

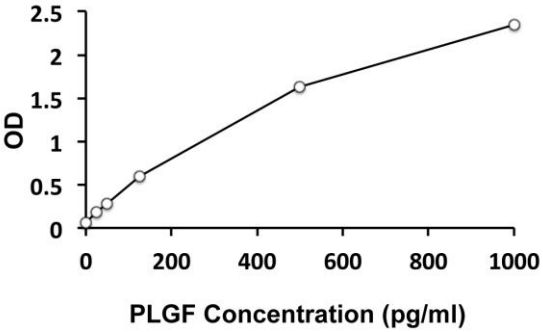
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of PLGF ranged from 1.06-1000 pg/ml. The mean MDD was <1.062 pg /ml.

Specificity

Less than 20% cross-reactivity with rhVEGF/PLGF, less than 0.07% cross-reactivity with rhFLT, rmPLGF-2, rhPDGF, and rhVEGF was observed.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 2.25% and inter-assay precision was 5.55%.

Recovery

87-105.5%