PGF (Human) ELISA Kit

Catalog Number KA0410

96 assays

Version: 03

Intended for research use only

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Introduction

Intended Use

For quantitive detection of human PLGF in sera, plasma, body fluids, tissue lysates or cell culture supernates.

Background

The protein, also called PGF, is 149 amino acids long and shares 53% identity with the platelet-derived growth factor-like region of human VPF. And the N-glycosylated PLGF protein is secreted into the medium and that it functions as a dimer[1]. The PLGF gene is mapped to 14q24-q31. There are 3 isoforms of PGF, designated PGF1, PGF2, and PGF3. Only PGF2 is able to bind heparin. Additionally, PGF regulates inter- and intramolecular cross-talk between the VEGF receptor tyrosine kinases FLT1 and FLK1[2]. It also can stimulate angiogenesis and collateral growth in ischemic heart and limb with at least a comparable efficiency to VEGF[3].

Principle of the Assay

The PGF (Human) ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human PLGF specific polyclonal antibody was precoated onto 96-well plates. The human PLGF specific detection polyclonal antibody was biotinylated. The test samples and biotinylated detection antibodies were added to the wells subsequently and then followed by washing with TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human PLGF amount of sample captured in plate.

General Information

Materials Supplied

List of component

Component	Amount
Lyophilized recombinant human PLGF standard	10 ng × 2
One plate precoated with anti-human PLGF antibody	96 wells
Sample diluent buffer	30 ml
Biotinylated anti-human PLGF antibody, dilution 1:100	130 µl
Antibody diluent buffer	12 ml
Avidin-Biotin-Peroxidase Complex (ABC), dilution 1:100	130 µl
ABC diluent buffer	12 ml
TMB color developing agent	10 ml
TMB stop solution	10 ml

Storage Instruction

Store at 4°C for frequent use, at -20°C for infrequent use. Avoid multiple freeze-thaw cycles. Four months at 4°C and eight months at -20°C.

Materials Required but Not Supplied

- ✓ Microplate reader in standard size.
- ✓ Automated plate washer.
- ✓ Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
- ✓ Clean tubes and Eppendorf tubes.
- ✓ Washing buffer (neutral PBS or TBS).

  Preparation of 0.01M TBS:

  Add 1.2g Tris, 8.5g NaCl; 450µl of purified acetic acid or 700µl of concentrated hydrochloric acid to 1000ml H_2O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

  Preparation of 0.01 M PBS:

  Add 8.5g sodium chloride, 1.4g Na_2HPO_4 and 0.2g NaH_2PO_4 to 1000ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

Precautions for Use

- ✓ Before using Kit, spin tubes and bring down all components to bottom of tube.
- ✓ Duplicate well assay was recommended for both standard and sample testing.
- ✓ Don't let 96-well plate dry, dry plate will inactivate active components on plate.
- ✓ In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre-warmed in 37°C for 30 min before using.

Assay Protocol

Reagent Preparation

- Reconstitution of the human PLGF standard: PLGF standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of PLGF standard (10ng per tube) are included in each kit. Use one tube for each experiment.
    a. 10,000pg/ml of human PLGF standard solution: Add 1 ml sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.
    b. 1000pg/ml of human PLGF standard solution: Add 0.1 ml of the above 10ng/ml PLGF standard solution into 0.9 ml sample diluent buffer and mix thoroughly.
    c. 500pg/ml→15.6pg/ml of human PLGF standard solutions: Label 6 Eppendorf tubes with 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.3pg/ml, 15.6pg/ml, respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the above 1000pg/ml PLGF standard solution into 1st tube and mix. Transfer 0.3 ml from 1st tube to 2nd tube and mix. Transfer 0.3 ml from 2nd tube to 3rd tube and mix, and so on.

  Note: The standard solutions are best used within 2 hours. The 10 ng/ml standard solution may be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.
- Preparation of biotinylated anti-human PLGF antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.
    a. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
    b. Biotinylated anti-human PLGF antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly.
- Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.
    a. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
    b. Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly.

Sample Preparation

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze thaw cycles.

- Cell culture supernate, tissue lysate or body fluids: Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C
- Serum: Allow the serum to clot in a serum separator tube (about 30 minutes) at room temperature. Centrifuge at approximately 1000 x g for 15 min. Analyze the serum immediately or aliquot and store

frozen at -20°C.

- Plasma: Collect plasma using heparin, EDTA, citrate as an anticoagulant. Centrifuge for 15 min at 1000 x g within 30 min of collection. Analyze immediately or aliquot and store frozen at -20°C.

Sample Dilution Guideline

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. The sample must be well mixed with the diluents buffer.

- ✓ High target protein concentration (10-100 ng/ml). The working dilution is 1:100. i.e. Add 1 µl sample into 99 µl sample diluent buffer.
- ✓ Medium target protein concentration (1-10 ng/ml). The working dilution is 1:10. i.e. Add 10 µl sample into 90 µl sample diluent buffer.
- ✓ Low target protein concentration (15.6-1000 pg/ml). The working dilution is 1:2. i.e. Add 50 µl sample to 50 µl sample diluent buffer.
- ✓ Very Low target protein concentration (≤ 15.6 pg/ml). No dilution necessary, or the working dilution is 1:2.

Assay Procedure

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard PLGF detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of PLGF amount in samples.

1. Aliquot 0.1 ml per well of the 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.3 pg/ml, 15.6 pg/ml human PLGF standard solutions into the precoated 96-well plate. Add 0.1 ml of the sample diluent buffer into the control well (Zero well). Add 0.1ml of each properly diluted sample of human sera, plasma, body fluids, tissue lysates or cell culture supernatants to each empty well. See “Sample Dilution Guideline” above for details. We recommend that each human PLGF standard solution and each sample is measured in duplicate.
2. Seal the plate with the cover and incubate at 37°C for 90 min.
3. Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
4. Add 0.1 ml of biotinylated anti-human PLGF antibody working solution into each well and incubate the plate at 37°C for 60 min.
5. Wash plate 3 times with 0.01 M TBS or 0.01 M PBS, and each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.(Plate Washing Method: Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of THREE washes. *Note: For automated*

washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the plate onto paper towels or other absorbent material.)

6. Add 0.1 ml of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min.
7. Wash plate 5 times with 0.01 M TBS or 0.01 M PBS, and each time let washing buffer stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 5 for plate washing method).
8. Add 90 µl of prepared TMB color developing agent into each well and incubate plate at 37°C in dark for 15-20 min *(Note: For reference only, the optimal incubation time should be determined by end user. And the shades of blue can be seen in the wells with the four most concentrated human PLGF standard solutions; the other wells show no obvious color).*
9. Add 0.1 ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450 nm in a microplate reader within 30 min after adding the stop solution.

Summary

1. Add samples and standards and incubate the plate at 37°C for 90 min. Do not wash.
2. Add biotinylated antibodies and incubate the plate at 37°C for 60 min. Wash plate 3 times with 0.01M TBS.
3. Add ABC working solution and incubate the plate at 37°C for 30 min. Wash plate 5 times with 0.01M TBS.
4. Add TMB color developing agent and incubate the plate at 37°C in dark for 15-20 min.
5. Add TMB stop solution and read.

Data Analysis

Calculation of Results

For calculation, (the relative $O.D._{450}$) = (the $O.D._{450}$ of each well) – (the $O.D._{450}$ of Zero well). The standard curve can be plotted as the relative $O.D._{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The human PLGF concentration of the samples can be interpolated from the standard curve.

Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

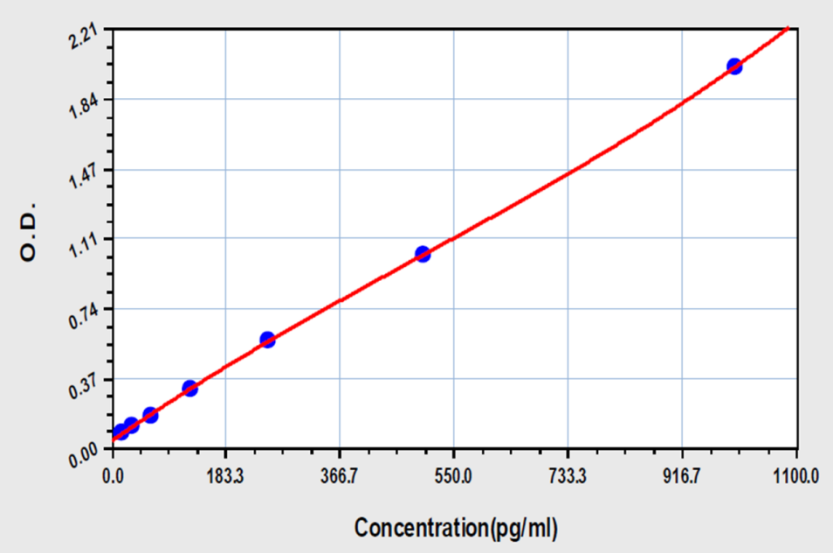
- Typical result

Typical Data Obtained from Human PLGF

Concentration	0.0pg/ml	15.6pg/ml	31.3pg/ml	62.5pg/ml	125pg/ml	250pg/ml	500pg/ml	1000pg/ml
O.D	0.042	0.092	0.121	0.174	0.319	0.570	1.023	2.010

(TMB reaction incubate at 37°C for 15 min)

PLGF (Human) ELISA Kit



This standard curve was generated for demonstration purpose only. A standard curve must be run with each assay.

Performance Characteristics

- Range
  15.6 - 1000 pg/ml

- Sensitivity
  < 1 pg/ml

- Specificity
  No detectable cross-reactivity with any other cytokine

Resources

References

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2. Autiero, M.; Waltenberger, J.; Communi, D.; Kranz, A.; Moons, L.; Lambrechts, D.; Kroll, J.; Plaisance, S.; De Mol, M.; Bono, F.; Kliche, S.; Fellbrich, G.; and 16 others : Role of PlGF in the intra- and intermolecular cross talk between the VEGF receptors Flt1 and Flk1. Nature Med. 9: 936-943, 2003.
3. PLGF stimulated angiogenesis and collateral growth in ischemic heart and limb with at least a comparable efficiency to VEGF. Luttun, A.; Tjwa, M.; Moons, L.; Wu, Y.; Angelillo-Scherrer, A.; Liao, F.; Nagy, J. A.; Hooper, A.; Priller, J.; De Klerck, B.; Compernolle, V.; Daci, E.; and 10 others : Revascularization of ischemic tissues by PlGF treatment, and inhibition of tumor angiogenesis, arthritis and atherosclerosis by anti-Flt1. Nature Med. 8: 831-840, 2002.

Plate Layout

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