Technical Manual

Human PGF (Placental Growth Factor) Superset Max ELISA Kit

- Catalogue Code: AEES02794
- Sandwich ELISA Kit
- Research Use Only

1. Description and Principle

The Assay Genie Human PGF (Placental Growth Factor) Superset Max ELISA Kit can assay for the specific analyte in the following samples: serum, blood, plasma, and other biological fluids.

2. Key features and Sample Types

Sensitivity: -

Detection Range: 31.25-2000 pg/mL

ELISA Type: Sandwich

Specificity: This kit recognizes Human PGF (Placental Growth Factor) Superset Max in samples. No significant cross-reactivity or interference between Human PGF (Placental Growth Factor) Superset Max and analogues was observed.

SUMMARY

1. Add 100µL Capture Antibody working solution. Incubate overnight at 2-8°C.
2. Discard the liquid, add 100µL standard or sample to the wells. Incubate for 90 min at 37°C.
3. Discard the liquid, immediately add 100µL Biotinylated Detection Ab working solution. Incubate for 60 min at 37°C
4. Aspirate and wash 3 times.
5. Add 100µL HRP Conjugate. Incubate for 30 min at 37°C.
6. Add 90µL Substrate Reagent. Incubate for 15 min at 37°C.
7. Add 50µL Stop Solution. Read at 450 nm immediately.
8. Calculation of results

3. Kit Contents

Product	Size	Cat. Code
Human PGF (Placental Growth Factor) Superset Max ELISA Kit	**96 assays**	**AEES02794**

Each kit contains reagents for 24/96 assays in a 24/96 well plate including:

Item	96Tx5	96Tx15	Dilution	Storage
Micro ELISA Plate (Dismountable)	5 plates	15 plates	-	-20°C, 12 months
Capture Ab	1 vial, 120 µL	1 vial, 350 µL	1/500-1/1000	
HRP Conjugate	1 vial, 120 µL	1 vial, 350 µL	1/500-1/1000	
Reference Standard	5 vials	5 vials	2000 pg /vial	-20°C (shading light), 12 months
Biotinylated Detection Ab	1 vial, 120 µL	1 vial, 150 µL	1/500-1/1000	4°C, 12 months
Plate Sealer	5 pieces	5 pieces	-	
Product Description	1 copy	1 copy	-	

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution. The volume of reagents in partial shipments is a little more than the volume marked on the label, please use accurate measuring equipment instead of directly pouring into the vial(s).

Other Reagents required

1. Ancillary Reagent Kit (SKU: AEES03043). The kit contains a full set of ancillary reagents to complete the 96T*5 ELISA assay.
2. Or refer to the following formula to prepare each universal reagent. (Note: The following formula only contains the basic component information of each reagent, which can be optimized according to the experimental requirements and results)
    - Coating Buffer: 1×CBS
    - Blocking Buffer: 1×PBS, Protective substance
    - Wash Buffer: 3% Tris
    - Standard & Sample Diluent: 1×PBS, Protective substance
    - Antibody & HRP conjugate Diluent: 1×PBS, Protective substance
    - Stop Solution: 5% sulfuric acid

Other Additional Materials required

1. Microplate reader with 450 nm wavelength filter
2. High-precision transfer pipette, EP tubes and disposable pipette tips
3. Incubator capable of maintaining 37°C
4. Deionized or distilled water
5. Absorbent paper

4. Shipping and Storage

An unopened kit can be stored at 4°C for 1 month. If the kit is not used within 1 month, store the items separately according to the vial labels.

5. Sample Preparation

Serum: Allow samples to clot for 1 hour at room temperature or overnight at 2-8℃before centrifugation for 20 min at 1000×g at 2-8℃. Collect the supernatant to carry out the assay.

Plasma: Collect plasma using EDTA-Na2 as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2-8℃ within 30 min of collection**.** Collect the supernatant to carry out the assay**.**

Recommended reagents for sample preparation: 10×EDTA Anticoagulant

Note for sample:

1) Tubes for blood collection should be disposable and be non-endotoxin. Samples with high hemolysis or much lipid are not suitable for ELISA assay.

2) Samples should be assayed within 7 days when stored at 2-8℃, otherwise samples must be divided up and stored at -20℃ (≤1 month) or -80℃ (≤3 months). Avoid repeated freeze-thaw cycles. Prior to assay, the frozen samples should be slowly thawed and centrifuged to remove precipitates.

3) Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

4) If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity. If a lysis buffer is used to prepare samples, there is a possibility of causing a deviation due to the introduced chemical substance.

5) Some recombinant protein may not be detected due to a mismatching with the coated antibody or detection antibody.

6. Dilution Method

Please predict the concentration range of samples in advance and determine the dilution ratio through preliminary experiments or technical support recommendations.

If your test sample needs dilution, please refer to the dilution method as follows:

For 100-fold dilution: One-step dilution. Add 5 µL sample to 495 µL sample diluent to yield 100-fold dilution.

For 1000-fold dilution: Two-step dilution. Add 5 µL sample to 95 µL sample diluent to yield 20-fold dilution, then add 5 µL 20-fold diluted sample to 245 µL sample diluent, after this, the neat sample has been diluted at 1000-fold successfully.

For 100000-fold dilution: Three-step dilution. Add 5 µL sample to 195 µL sample diluent to yield 40-fold dilution, then add 5 µL 40-fold diluted sample to 245 µL sample diluent to yield 50-fold dilution, and finally add 5 µL 2000-fold diluted sample to 245 µL sample diluent, after this, the neat sample has been diluted at 100000-fold successfully.

7. Protocol

1. Bring all reagents to room temperature (18~25°C) before use. Follow the microplate reader manual for set-up and preheat it for 15 min before OD measurement.

2. Micro ELISA Plate:

a) The capture antibody was diluted to the working concentration using the ELISA Plate Coating Buffer(1×) (1/500-1/1000 fold dilution is recommended).

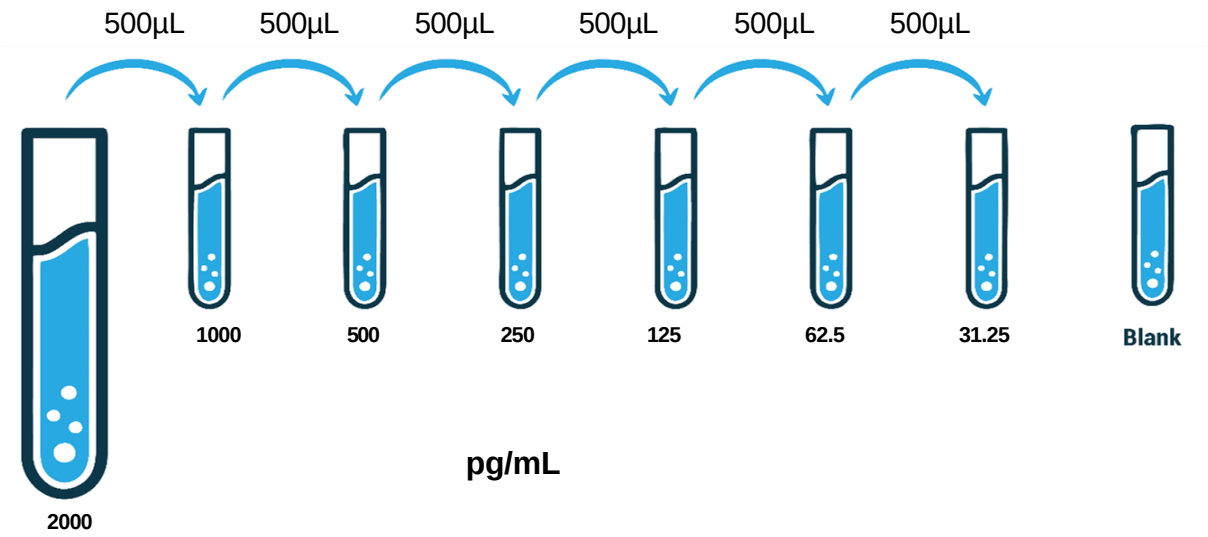
b) Take out the Micro ELISA pre-Plate, add 100 μL of capture antibody working solution to each well. Cover the plate with the sealer provided in the kit. Incubate overnight at 2-8℃.

c) Decant the liquid from each well, do not wash. Add 200 μL of ELISA Plate Blocking Buffer to each well. Cover the plate with the sealer. Incubate for 1 hour at 37℃.

d) Decant the liquid from each well, do not wash, and the plate is ready for sample addition. Or the plate was dried at 37℃ for 30 min. The dried plate can be stored at -20℃ for 6 months after sealing with desiccant.

3. Standard working solution: Centrifuge the standard at 10,000×g for 1 min. Add 1 mL of Reference Standard & Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 2000 pg/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 1000 pg/mL. Note: the last tube is regarded as a blank. Don't pipette solution into it from the former tube.

Dilution method: Take 7 EP tubes, add 500μL of Reference Standard & Sample Diluent to each tube. Pipette 500μL of the 2000 pg/mL working solution to the first tube and mix up to produce a 1000 pg/mL working solution. Pipette 500μL of the solution from the former tube into the latter one according to these steps. The illustration below is for reference.

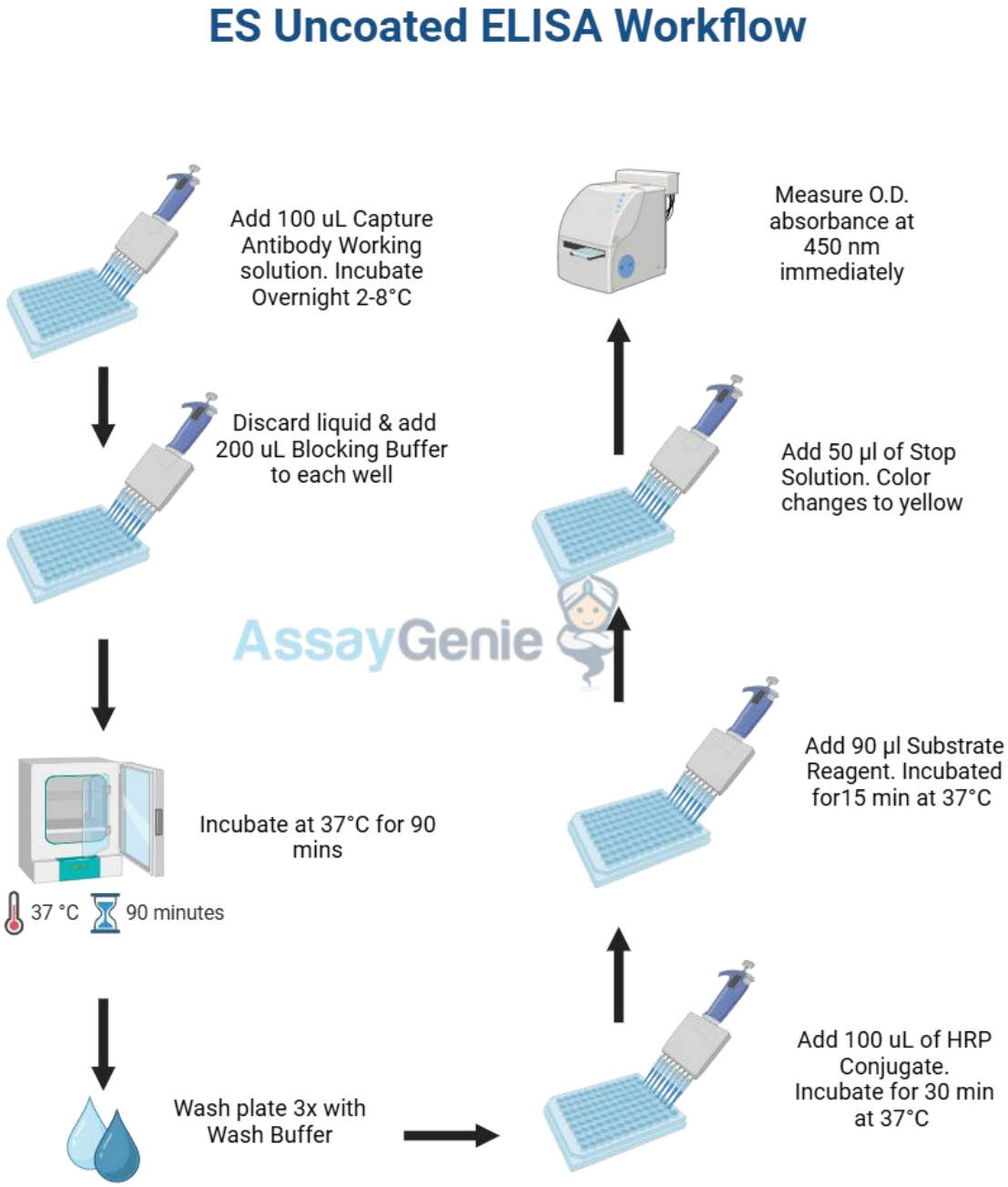


4. Biotinylated Detection Ab working solution: Calculate the required amount before the experiment (100 µL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated Biotinylated Detection Ab at 800×g for 1 min, then dilute the Biotinylated Detection Ab to working solution with Biotinylated Antibody Diluent (1/500-1/1000 fold dilution is recommended). The working solution should be prepared just before use.

5. Concentrated HRP Conjugate working solution: HRP Conjugate is HRP conjugated avidin. Calculate the required amount before the experiment (100 µL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the HRP Conjugate at 800×g for 1 min, then dilute the HRP Conjugate to working solution with HRP Conjugate Diluent (1/500-1/1000 fold dilution is recommended). The working solution should be prepared just before use.

6. Wash Buffer: Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40℃ water bath and mix it gently until the crystals have completely dissolved. For same day use only.

8. Workflow Overview



9. Assay procedure

1. Determine wells for diluted standard, blank and sample. Add 100 µL each dilution of standard, blank and sample into the appropriate wells (It is recommended that all samples and standards be assayed in duplicate. It is recommended to determine the dilution ratio of samples through preliminary experiments or technical support recommendations). Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37℃. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.

2. Decant the liquid from each well, do not wash. Immediately add 100 µL of Biotinylated Detection Ab working solution to each well. Cover the plate with a new sealer. Incubate for 1 hour at 37°C.

3. Decant the solution from each well, add 350 µL of wash buffer to each well. Soak for 1 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps. Make the tested strips in use immediately after the wash step. Do not allow wells to be dry.

4. Add 100 µL of HRP Conjugate working solution to each well. Cover the plate with a new sealer. Incubate for 30 min at 37°C.

5. Decant the solution from each well, repeat the wash process for 5 times as conducted in step 3.

6. Add 90 µL of Substrate Reagent to each well. Cover the plate with a new sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min. Preheat the Microplate Reader for about 15 min before OD measurement.

7. Add 50 µL of Stop Solution to each well. Note: adding the stop solution should be done in the same order as the substrate solution.

8. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

10. Data analysis

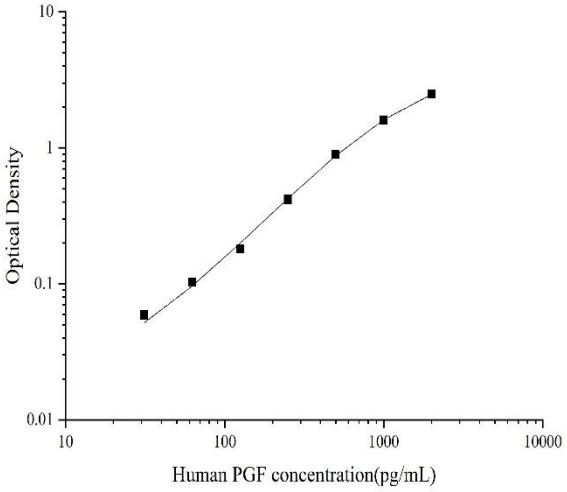
Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Plot a four-parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis.

If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it with an appropriate dilution. The actual concentration is the calculated concentration multiplied by the dilution factor.

Typical data

As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should generate a standard curve for each experiment. Typical standard curve and data is provided below (for reference only).

Concentration (pg/mL)	OD	Corrected OD
2000	2.516	2.423
1000	1.71	1.617
500	0.979	0.886
250	0.545	0.452
125	0.329	0.236
62.5	0.199	0.106
31.25	0.149	0.056
0	0.093	-



Specificity

This kit recognizes Human PGF (Placental Growth Factor) Superset Max in samples. No significant cross-reactivity or interference between Human PGF (Placental Growth Factor) Superset Max and analogues was observed.

11. Important General Notes:

Problem	Causes	Solutions
Poor standard curve	Inaccurate pipetting	Check pipettes.
	Improper standard dilution	Centrifuge the standard vial and ensure contents are dissolved thoroughly.
	Wells are not completely aspirated	Completely aspirate wells in between steps.
Low signal	Insufficient incubation time	Ensure sufficient incubation time.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrate to room temperature before use.
	Inadequate reagent volumes	Check pipettes and ensure correct preparation.
	Improper dilution	
	HRP conjugate inactive or TMB failure	Mix HRP conjugate and TMB = rapid color change.
Deep color but low value	Plate reader setting is not optimal	Verify the wavelength and filter setting on the Microplate reader.
		Pre-heat Microplate Reader.
Large CV	Inaccurate pipetting	Check pipettes.
High background	Concentration of target protein is too high	Use recommended dilution factor.
	Plate is insufficiently washed	Review the manual for proper washing procedure. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Prepare fresh wash buffer.
Low sensitivity	Improper storage of the ELISA kit	All the reagents should be stored according to the instructions.
	Stop solution is not added	Stop solution should be added to each well before measurement.

Additional Notes:

1. Please wear lab coats, eye goggles and latex gloves for protection. Perform the experiment following the national safety guidelines for biological laboratories, especially when using blood samples or other bodily fluids.

2. A freshly opened ELISA Plate may appear to have a water-like substance, this is normal and will not have any impact on the experimental results.

3. Do not reuse the reconstituted standard, biotinylated detection Ab working solution, concentrated HRP conjugate working solution. The unspent undiluted concentrated biotinylated detection Ab (100×) and other stock solutions should be stored according to the storage conditions in the above table.

4. The microplate reader should have a 450(±10 nm) filter installed and a detector that can detect this wavelength. The optical density should be within 0~3.5.

5. Do not mix or use components from other lots.

6. Change pipette tips in between adding standards, sample additions and reagent additions. Also, use separate reservoirs for each reagent.

Declaration

1. Limited by current scientific technology, we can't conduct comprehensive identification and analysis on all the raw materials provided [So, there might be some qualitative and technical risks for users using the kit].

2. The final experimental results will be closely related to the validity of products, operational skills and the experimental environment. Please make sure that sufficient samples are available.

3. To get the best results, please only use the reagents supplied with this kit and strictly comply with the instructions.

4. Incorrect results may occur from incorrect reagent preparation and loading, as well as incorrect parameter settings of the Micro-plate reader. Please read the instructions carefully and adjust the instrument prior to the experiment.

5. Each kit passes a strict QC procedure. However, results from end users might be inconsistent with our data due to some variables such as transportation conditions, different lab equipment, and so on. Intra-assay variance among kits from different batches might also arise.

Notes:

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Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

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