Technical Manual

Human PAF(Platelet Activating Factor) ELISA Kit

- Catalogue Code: HUFI03177
- Sandwich ELISA Kit
- Research Use Only

Contents

1. Key features and Sample Types

Aliases:

Platelet Activating Factor

Uniprot:

Detection method:

Sandwich, Double Antibody

Sample Type:

Serum, Plasma and other biological fluids

Reactivity:

Human

Range:

0.156-10ng/ml

Sensitivity:

0.094ng/ml

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Please store this ELISA Kit at 4°C. Date of expiration will be on the ELISA Box label.

3. Description and Principle

The Assay Genie Sandwich ELISA kit is a highly sensitive assay for the quantitative measurement of a specific analyte in the following samples: serum, blood, plasma, cell culture supernatant and other related supernatants and tissues.

How do our ELISA kits work?

The Assay Genie (enzyme-linked immunosorbent assays) assay kits are designed for the quantitative measurement of analytes in a wide variety of samples. As today's scientists demand premium quality, consistent data, Assay Genie have developed a range of sensitive, fast and reliable ELISA kit assays to meet and exceed those demands. Our assay kits use a quantitative sandwich ELISA technique and each kit comes with highly specific antibodies pre-coated onto a 96-well microtiter plate.

At Assay Genie we understand the need for speed! Therefore, we have developed an ultrafast protocol for rapid results. Once you have prepared and plated your samples, blanks and standards, you simply incubate with the specific biotin-conjugated primary antibody and Avidin conjugated Horseradish Peroxidase (HRP). After plate washing and addition of the TMB (3,3',5,5'-Tetramethylbenzidine) solution, the appearance of a blue colour is detected due to an enzymatic reaction catalysed by HRP. Next the addition of the Stop Solution terminates the HRP reaction and the blue colour turns yellow with the signal intensity measured on a plate reader at 450nm. The amount of bound analyte is proportional to the signal generated by the reaction meaning the kit assay gives you a quantitative measurement of the analyte in your samples.

4. Kit Contents

Each kit contains reagents for either 48 or 96 assays including:

No.	Component	48-Well Kit	96-Well Kit	Storage
1	ELISA Microplate (dismountable)	8 x 6	8 x 12	2-8°C/-20°C
2	Lyophilized Standard	1 vial	2 vials	2-8°C/-20°C
3	Sample Dilution Buffer	10 mL	20 mL	2-8°C
4	Biotin-labeled Antibody (Concentrated)	60 uL	120 uL	2-8°C (Avoid Direct Light)
5	Antibody Dilution Buffer	5 mL	10 mL	2-8°C
6	HRP-Streptavidin Conjugate (SABC)	60 uL	120 uL	2-8°C (Avoid Direct Light)
7	SABC Dilution Buffer	5 mL	10 mL	2-8°C
8	TMB Substrate	5 mL	10 mL	2-8°C (Avoid direct light)
9	Stop Solution	5ml	10ml	2-8°C
10	Wash Buffer (25x)	15 mL	30 mL	2-8°C
11	Plate Sealer	3 pieces	5 pieces	
12	Manual	1	1	

Additional materials required:

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 µl to 1 ml volumes.
3. Adjustable 1-25 ml pipettes for reagent preparation.
4. 100 ml and 1 liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.
7. Log-log graph paper or computer and software for ELISA data analysis.
8. Tubes to prepare standard or sample dilutions.

Precautions:

1. To identify the concentration of your target, a pilot experiment using standards and a small number of samples is recommended.

2. Ensure unopened and unused plate is kept dry to avoid contamination.

3. Before using the kit, centrifuge tubes to spin down standard & antibodies.

4. Avoid light for storage of TMB reagents.

5. Wash steps are critical for the success of the assay, deviations from wash steps may cause false positives and result in a high background.

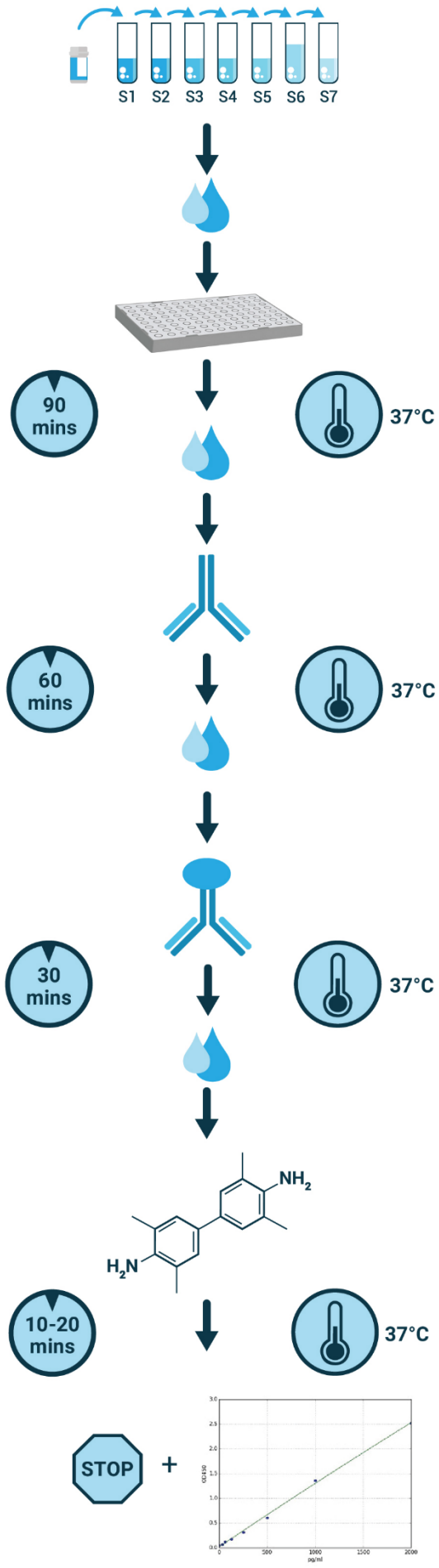
6. Duplicate wells are recommended for both standard and sample testing.

7. Do not let the microplate wells dry during assay. Dry plates will inactivate active components.

8. Do not reuse tips and tubes to avoid cross contamination.

9. Avoid using the reagents from different batches together.

5. Workflow Overview



Prepare plate standards and reagents

Wash plate x2

Add 100µl of samples, standards and controls

Wash plate x 2

Add 100µl Biotin-labeled Antibody working solution

Wash plate x3

Add 100µl SABC working solution

Wash plate x 5

Add 90µl TMB Substrate

Add 50µl STOP solution and read absorbance @ 450nm

6. Sample Preparation

General considerations: According to best practices, extract protein & perform the experiment as soon as possible after sample collection. Alternatively, store the extracts at the designated temperature (-20°C/-80°C). For optimal results, avoid repeated freeze-thaw cycles.

Serum: If using serum separator tubes, allow samples to clot for 30 minutes at room temperature. Centrifuge for 20 minutes at 1,000x g. Collect the serum fraction and assay promptly or aliquot and store the samples at -80°C. Avoid multiple freeze-thaw cycles.

If serum separator tubes are not being used, allow samples to clot overnight at 2-8°C. Centrifuge for 20 minutes at 1,000x g. Remove serum and assay promptly or aliquot and store the samples at -80°C. Avoid multiple freeze-thaw cycles.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples at 4°C for 15 mins at 1000 × g within 30 mins of collection. Collect the plasma fraction and assay promptly or aliquot and store the samples at -80°C. Avoid multiple freeze-thaw cycles.

Note: Over haemolyzed samples are not suitable for use with this kit.

Cell culture supernatant: Collect the cell culture media by pipette, followed by centrifugation at 4°C for 20 mins at 1000 x g. Collect the clear supernatant and assay immediately.

Cell lysates: Commercial RIPA kits are recommended for preparation of cell lysate samples. For 2 x 10^6 cells, lyse cells in 0.5 mL RIPA buffer, removing DNA content. Determine the protein concentration using a BCA assay kit (BN01031) with each sample concentration being ≤ 300µg for analysis.

Tissue Homogenates: Rinse tissue with 1X PBS to remove excess blood. Mince tissue after weighing it and homogenize in PBS (the volume depends on the weight of the tissue. 9mL PBS (including protease inhibitors) would be appropriate for 1 gram of tissue. To further lyse cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000 x g and the supernatant is removed for assaying. The total protein concentration was determined by BCA kit and the total protein concentration of each pore sample should not exceed 0.3mg.

Notes

Samples stored at 2-8°C should be used within 5 days, samples stored at -20°C should be assayed within 1 month and samples stored at -80°C should be assayed within 2 months to reduce the loss of bioactivity. Avoid multiple freeze-thaw cycles. Hemolysed samples are not suitable for this assay.

7. Standard and Reagent Preparation

Manual Washing

Discard the solution in the plate without touching the side of the wells. Clap the plate on absorbent filter paper or other absorbent material. Fill each well completely with 350µl wash buffer and soak for 1 to 2 mins, then aspirate contents from the plate, and clap the plate on absorbent filter paper or other absorbent material. Repeat this procedure for the designated number of washes.

Automated Washing

Aspirate all wells, then wash plate with 350µl wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter paper or other absorbent material. It is recommended that the washer is set for a soaking time of 1 minute (Note: set the height of the needles; be sure the fluid can be taken up completely).

Sample Dilution Guidelines

Determine the concentration of the target protein in the test sample and then select the optimal dilution factor to ensure the target protein concentration falls within the optimal detection range of the kit. Dilute the samples with the dilution buffer provided with the kit. Several dilution tests may be required to achieve the optimal results. The test samples must be well mixed with the dilution buffer. Standard and sample dilution should be performed before starting the experiment.

Note: Matrix components in the sample will affect test results, samples need to be diluted at least ½ with Sample Dilution Buffer before testing.

Reagent Preparation

Bring all reagents and samples to room temperature 20 minutes before use.

1. Wash Buffer:

Dilute 30 ml (15 ml for 48T) of Concentrated Wash Buffer into 750 ml (375 ml for 48T) of Wash Buffer with deionized or distilled water. Store unused solution at 4°C. If crystals have formed in the concentrate, warm at 40°C in water bath (Heating temperature should not exceed 50°C) and mix gently until crystals have completely dissolved. The solution should be cooled to room temperature before use.

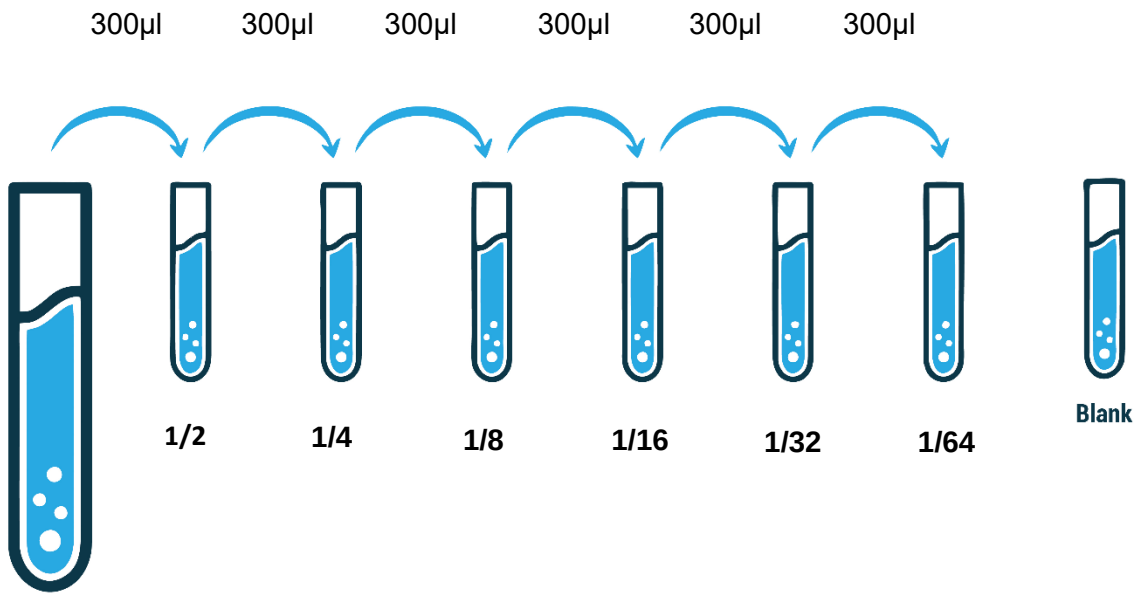
2. Standard Dilution:

1). Add 1ml of Sample dilution buffer into one Standard tube (labelled as Stock Solution), keep the tube at room temperature for 10 min and mix thoroughly.

Note: If the standard vial concentration is different to the highest value in the range (please see page 3), please dilute using sample buffer to match highest range value to create stock solution.

2). Label 7 Eppendorf tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Aliquot 300µl of the Sample dilution buffer into each tube. Add 300µl of the above (Stock Solution) standard solution into 1st tube and mix thoroughly. Transfer 300µl from 1st tube to 2nd tube and mix thoroughly. Transfer 300µl from 2nd tube to 3rd tube and mix thoroughly, and so on. Sample dilution buffer is used as blank control.

DILUTION SERIES



Note: The standard solutions are best used within 2 hours. The standard solution series should be kept at 4°C for up to 12 hours. Or store at -20 °C for up to 48 hours. Avoid repeated freeze-thaw cycles.

3. Preparation of biotin-labeled Antibody working solution:

Prepare within 1 hour before the experiment.

1. Calculate the total volume of the working solution: 100µl / well × quantity of wells (Allow 100-200µl more than the total volume).
2. Dilute the Biotin-detection Antibody with antibody dilution buffer at 1:100 and mix thoroughly (i.e. Add 1µl of Biotin-labeled Antibody into 99µl of Antibody Dilution Buffer.)

4. Preparation of HRP-Streptavidin Conjugate (SABC) working solution:

Prepare within 30 minutes of starting the experiment.

1. Calculate the total volume of the working solution: 100µl / well × quantity of wells. (Allow 100-200µl more than the total volume)
2. Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly. (i.e. Add 1µl of SABC into 99µl of SABC dilution buffer.)

8. Assay Procedure

Before adding to the wells, equilibrate the TMB substrate for at least 30 mins at 37°C. When diluting samples and reagents, ensure they are mixed completely and evenly. It is recommended to plot a standard curve for each test.

1. Set standard, **test sample (diluted at least ½ with Sample Dilution Buffer)** and control (zero) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate. **Wash plate 2 times before adding standard, sample and control (blank) wells.**
2. **Standards:** Aliquot 100µl of standard solutions into the standard wells.
3. **Dilution Buffer:** Add 100µl of Sample dilution buffer into the control (blank) well.
4. **Samples:** Add 100µl of properly diluted sample into test sample wells.
5. **Incubate:** Seal the plate with a cover and incubate at 37 °C for 90 mins.
6. **Wash:** Remove the cover, aspirate the liquid from the plate and wash plate 2 times with Wash Buffer. **Do NOT let the wells dry completely at any time.**
7. **Biotin-labeled Antibody:** Add 100µl of Biotin-labelled antibody working solution to the bottom of each well (standard, test sample & zero wells) without touching the side walls.
8. **Incubate:** Seal the plate with a cover and incubate at 37°C for 60 mins.

9. **Wash:** Remove the cover, and wash plate 3 times with Wash buffer. **Let the Wash Buffer stay in the wells for 1-2 minutes for each wash.**
10. **HRP-Streptavidin Conjugate (SABC):** Add 100µl of SABC working solution into each well, cover the plate and incubate at 37°C for 30 mins.
11. **Wash:** Remove the cover and wash plate 5 times with Wash buffer. **Let the Wash Buffer stay in the wells for 1-2 minutes for each wash.**
12. **TMB Substrate:** Add 90 µl of TMB substrate into each well, cover the plate and incubate at 37°C in dark for 10-20 mins. (Note: This incubation time is for reference only, the optimal time should be determined by the end-user.) As soon as a blue colour develops in the first 3-4 wells (with most concentrated standards) and the other wells show no obvious colour, terminate the reaction by moving to Step 13.
13. **Stop Solution:** Add 50µl of Stop solution into each well and mix thoroughly. The colour changes into yellow immediately.
14. **OD Measurement:** Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the stop solution.

9. Data Analysis

Calculate using the following equation:

The relative O.D.450 = (the O.D.450 of each well) – (the O.D.450 of blank well)

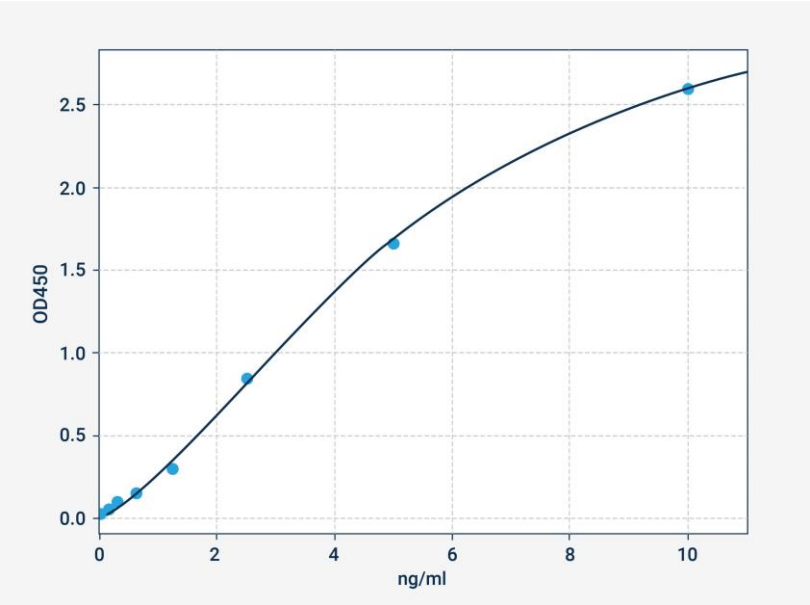
The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The concentration of the samples can be determined from the standard curve. It is recommended to use professional software such as curve expert 1.3 or 1.4.

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

10. Typical Data & Standard Curve

Standard Curve

Results of a typical standard run of a ELISA kit are shown below. This standard curve was generated at our lab for demonstration purpose only. Each user should obtain their own standard curve as per experiment.



Specificity

This assay has high sensitivity and excellent specificity for detection of Human PAF. No significant cross-reactivity or interference between Human PAF and analogues was observed.

Note: Limited by current skills and knowledge, it is difficult for us to complete the cross-reactivity detection between Human PAF and all the analogues, therefore, cross reaction may still exist.

Recovery

Matrices listed below were spiked with a certain level of Human PAF and the recovery rates were calculated by comparing the measured value to the expected amount of Human PAF in the samples.

Matrix	Recovery range(%)	Average(%)
serum(n=5)	86-101	94
EDTA plasma(n=5)	87-105	98
heparin plasma(n=5)	90-105	97

Linearity

The linearity of the kit was assayed by testing the samples spiked with appropriate concentration of Human PAF and their serial dilutions.

Sample	1:2	1:4	1:8
serum(n=5)	95-101%	88-104%	86-101%
EDTA plasma(n=5)	82-92%	82-93%	84-96%
heparin plasma(n=5)	91-99%	81-99%	86-98%

Precision

- **Intra-Assay:** CV<8%
- **Inter-Assay:** CV<10%

Stability

The stability of the Human PAF ELISA Kit is determined by the loss rate of activity. The loss rate of this kit is less than 10% within the expiration date under appropriate storage conditions.

Standard (n=5)	**37°C for 1 month**	**4°C for 6 months**
Average (%)	80	95-100

To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end**.**

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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