Platelet Activating Factor (PAF) ELISA Kit

rev 10/19

(Catalog # E4631-100, 100 assays, Store at 4°C)

I. Introduction:

Platelet-activating factor (PAF) is a potent phospholipid activator and mediator of many leukocyte functions, platelet aggregation and degranulation, inflammation, and anaphylaxis. It is also involved in changes to vascular permeability, the oxidative burst, chemotaxis of leukocytes, as well as augmentation of arachidonic acid metabolism in phagocytes. BioVision's PAF ELISA kit is a competitive ELISA assay for the quantitative measurement of PAF in serum, plasma and cell culture supernatants.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of PAF in samples.
Detection Range: 0.156 - 10 ng/ml
Sensitivity: < 0.094 ng/ml
Assay Precision: Intra-Assay: CV < 8%; Inter-Assay: CV < 10% (CV (%) = SD/mean X 100)
No significant cross-reactivity or interference between PAF and analogues was observed.

III. Sample Type:

Serum, plasma, tissue homogenates and other biological fluids

IV. Kit Contents:

Components	E4631-100	Part No.
Micro ELISA Plate	8 X 12 strips	E4631-100-1
Lyophilized Standard	2 vials	E4631-100-2
Sample / Standard dilution buffer	20 ml	E4631-100-3
Biotin- detection antibody (Concentrated)	60 µl	E4631-100-4
Antibody dilution buffer	10 ml	E4631-100-5
HRP-Streptavidin Conjugate (SABC) (Avoid light)	120 µl	E4631-100-6
SABC dilution buffer	10 ml	E4631-100-7
TMB substrate (Avoid light)	10 ml	E4631-100-8
Stop Solution	10 ml	E4631-100-9
Wash buffer (25X)	30 ml	E4631-100-10
Plate sealers	5	E4631-100-11

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- 37°C incubator

VI. Storage and Handling:

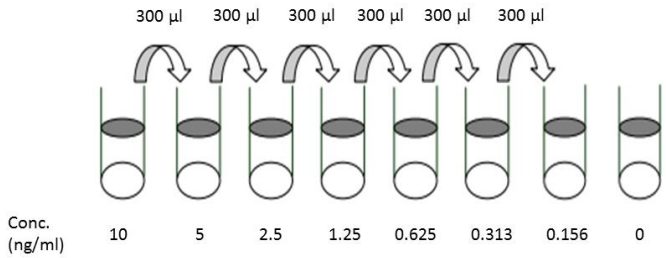
The entire kit may be stored at 4°C for up to 6 months from the date of shipment.

VII. Reagent and Sample Preparation:

Note: Prepare reagents within 30 minutes before the experiment. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Biotin- detection antibody working solution**: Calculate the total volume of the working solution: 0.05 ml / well × quantity of wells with additional 0.1 - 0.2 ml of the total volume. Dilute the Biotin- detection antibody with Antibody dilution buffer at 1:100 and mix thoroughly.
2. **HRP-Streptavidin Conjugate (SABC)**: Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells with additional 0.1 - 0.2 ml of the total volume. Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly.
3. **Wash Buffer**: Dilute 30 mL of Concentrated Wash Buffer into 750 mL of Wash Buffer with deionized or distilled water. Put unused solution back at 4°C. If crystals have formed in the concentrate, warm it with 40°C water bath and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.
4. **Standard Preparation:**

- Reconstitute the lyophilized PAF standard by adding 1 ml of Standard/Sample Dilution Buffer to make the 10 ng/ml standard stock solution. Use within 2 hours after reconstituting.
- Allow solution to sit at room temperature for 10 minutes, then gently vortex to mix completely.
- Prepare 0.6 ml of 5 ng/ml top standard by adding 0.3 ml of the above stock solution in 0.3 ml of Standard/Sample Dilution Buffer. Perform 2-fold serial dilutions of the top standards to make the standard curve within the range of this assay.
- Suggested standard points are: 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156, 0 ng/ml



5. **Sample Preparation:**

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Note: Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles.

- **Serum**: Coagulate the serum for 2 hour at room temperature or overnight at 4°C. Centrifuge at approximately 1000×g for 20 min. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.
- **Plasma**: Collect plasma using EDTA-Na_2 as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2 - 8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.
- **Tissue homogenates**: Rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess hemolysis blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS (the volume depends on the weight of the tissue. 9 mL PBS would be appropriate for 1 g of tissue. Some protease inhibitor is recommended to add into the PBS.) with a glass homogenizer on ice. To further break the cells, 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×g to retrieve the supernatant.
- **Cell culture supernatant**: Centrifuge supernatant for 20 minutes to remove insoluble impurity and cell debris at 1000×g at 2 - 8°C. Collect the clear supernatant and carry out the assay immediately or aliquot and store at -20°C.
- **Other biological fluids:** Centrifuge samples for 20 min at 1000×g at 4°C. Collect the supernatant and carry out the assay immediately.
- End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit.

VIII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay. It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VII.
2. Wash plate 2 times with **1X Wash Solution** before adding standard, sample and control wells.
3. Add 50 µl of each **standards** or **samples** into appropriate wells. Immediately add 50 µL Biotin-labeled Antibody Working Solution into each well. Cover well and incubate for 45 mins at 37ºC.
4. Remove the cover and wash 3 times with **1X Wash Solution**. Wash by filling each well with Wash Buffer (350 µl) using a multi-channel pipette or autowasher. Let it soak for 1-2 minutes, and then remove all residual wash-liquid from the wells by aspiration. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Clap the plate on absorbent filter papers or other absorbent materials.
5. Add 100 µl of **SABC working solution** into each well, cover the plate and incubate at 37ºC for 30 min.
6. Discard the solution and wash 5 times with **1X Wash Solution** as step 6.
7. Add 90 µl of **TMB substrate** into each well, cover the plate and incubate at 37 ºC in dark within 15-20 min. The reaction time can be shortened or extended according to the actual color change, but not more than 30minutes. Terminate the reaction when apparent gradient appeared in standard wells.
   Add 50 µl of **Stop Solution** to each well. Read result at 450 nm immediately after adding the stop solution.

IX. CALCULATION:

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 PAF concentration of the samples can be interpolated from the standard curve. If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

X	ng/ml	0	0.156	0.312	0.625	1.25	2.5	5	10
Y	OD450	2.372	1.554	1.109	0.835	0.584	0.408	0.268	0.204

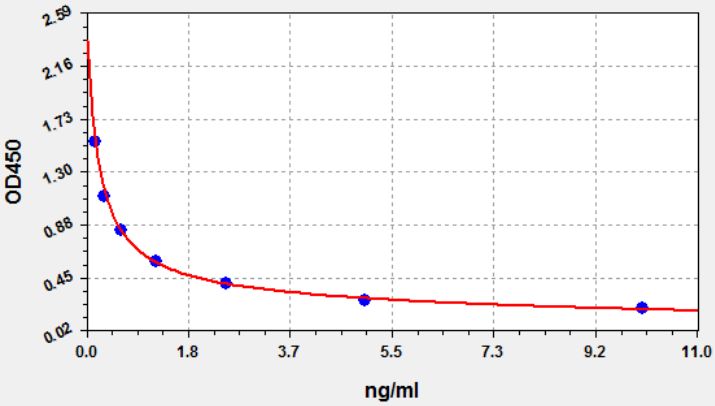

Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

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X. RECOVERY:

Matrix	Recovery range (%)	Average (%)
serum(n=5)	91-105	100
EDTA plasma(n=5)	86-101	93
heparin plasma(n=5)	90-101	95

XI. LINEARITY:

Sample	1:2	1:4	1:8	1:16
serum(n=5)	86-99%	92-105%	88-104%	85-104%
EDTA plasma(n=5)	83-99%	82-101%	90-101%	85-100%
heparin plasma(n=5)	81-99%	82-98%	85-100%	80-97%

XII. Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level PAF were tested 20 times on one plate, respectively.
Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level PAF were tested on 3 different plates, 8 replicates in each plate.

CV (%) = SD/meanX100
Intra-Assay: CV<8%
Inter-Assay: CV<10%

XIII. RELATED PRODUCTS:

- PAF Acetylhydrolase (PAF-AH) Inhibitor Screening Kit (Colorimetric) (K766)
- PAF Acetylhydrolase Activity Assay Kit (Colorimetric) (K765)
- Paf1 polyclonal antibody (6831)
- Acetylcholinesterase Activity Colorimetric Assay Kit (K764)
- Myeloperoxidase (MPO) Colorimetric Assay Kit (K744)

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