ab287801 – Platelet Activating Factor ELISA Kit

For the quantitative in vitro determination of Platelet Activating Factor in serum, plasma and cell culture supernatants.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab287801

Storage and Stability

The entire kit may be stored at 4°C in dark for up to 6 months from the date of shipment. Avoid freeze-thaw cycles.

Materials Supplied

Item	Quantity	Storage Condition
Micro ELISA Strip-plate	8 x 12 strips	4°C
Lypholized Standard	2 vial	4°C
Sample / Standard Dilution buffer	20 mL	4°C
Biotin-detection antibody (Concentrated)	60 µL	4°C
Antibody Dilution buffer	10 mL	4°C
HRP-Streptavidin Conjugate (SABC)	120 µL	4°C
SABC dilution buffer	10 mL	4°C
TMB Substrate	10 mL	4°C
Stop Solution	10 mL	4°C
25X Wash buffer	30 mL	4°C
Plate sealers	5	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

Reagent Preparation

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

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.

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.

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

Standard Preparation

1) 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.
2) Allow solution to sit at room temperature for 10 minutes, then gently vortex to mix completely.
3) 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

- 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-Na2 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

Δ Note: *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.*

Assay Procedure

- 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 for each assay.

1) Prepare all reagents, samples and standards as instructed (see above)
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.
4) Cover well and incubate for 45 mins at 37°C.
5) 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.
6) Add 100 µl of SABC working solution into each well, cover the plate and incubate at 37°C for 30 min.
7) Discard the solution and wash 5 times with 1X Wash Solution as step 5.
8) Add 90 µl of TMB substrate into each well, cover the plate and incubate at 37 °C 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

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.

Technical Support

For all technical or commercial enquiries please go to:
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