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PAF Acetylhydrolase Inhibitor Screening Kit (Colorimetric)

rev 11/20

(Catalog # K766-100; 100 assays; Store at -20 °C)

I. Introduction:

Platelet-Activating Factor (PAF or 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine) is an important phospholipid mediator, which has diverse biological activities. PAF is synthesized and secreted by a variety of cells such as mast cells, monocytes, macrophages etc. Up-regulated PAF signaling can cause pathological inflammation and also has been found to be responsible for sepsis, shock, and traumatic injury. PAF-Acetylhydrolase (PAF-AH or 1-alkyl-2-acetylglycerophosphocholine esterase or Lipoprotein-associated Phospholipase A_2 or Lp-PLA_2) (EC 3.1.1.47) hydrolyzes PAF by removing acetyl group at the sn-2 position and converts PAF into biologically inactive form, lyso-PAF. PAF-AH has two forms: extracellular and intracellular that shares some similarities. In human, PAF-AH deficiency leads to severe asthma, thus development of novel and specific inhibitors of PAF-AH is critical for therapeutic purposes. In **BioVision's PAF-AH Inhibitor Screening Kit,** PAF-AH hydrolyzes the acetyl thioester bond at sn-2 position of substrate and free thiols are detected using DTNB. In the presence of PAF-AH inhibitor, the reaction is impeded. The PAF-AH Inhibitor Control is included to compare the efficacy of test inhibitors. The assay is high-throughput adaptable and can be finished in less than 1 hr.

$$\text{PAF-AH Substrate + DTNB} \xrightarrow{\textbf{PAF-AH}} \text{Lyso- PAF + Color Product (OD 412 nm)}$$

$$\text{PAF-AH Substrate + DTNB + PAF-AH Inhibitor} \xrightarrow{\text{PAF-AH}} \text{Decreased Color Detection (OD 412 nm)}$$

II. Application:

- Screening/studying/characterizing potential PAF-AH inhibitors

III. Kit Contents:

Components	K766-100	Cap Color	Part Number
PAF-AH Assay Buffer	50 ml	NM	K766-100-1
DTNB (in DMSO)	100 µl	Red	K766-100-2
PAF-AH Substrate (in EtOH)	100 µl	Blue	K766-100-3
PAF-AH Enzyme	100 µl	Purple	K766-100-4
Inhibitor (in DMSO)	40 µl	Black	K766-100-5

IV. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer

V. Storage Conditions and Reagent Preparation:

Store kit at -20 °C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

- **PAF-AH Assay Buffer:** Bring to room temperature (RT) before use.
- **DTNB (in DMSO):** Before use, thaw at RT. Store at -20 °C.
- **PAF-AH Substrate (in EtOH):** Evaporate ethanol from PAF-AH Substrate vial (e.g. Use a gentle stream of nitrogen gas). Reconstitute with 220 µl PAF-AH Assay Buffer. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.
- **PAF-AH Enzyme:** Divide into aliquots and store at -70 °C. Avoid repeated freeze and thaw cycles. Keep on ice while in use. Use within two months.
- **Inhibitor (in DMSO):** Bring to RT before use.

VI. PAF-AH Inhibitor Screening Protocol:

1. **Enzyme Solution Preparation:** Before the assay, dilute PAF-AH Enzyme 1:5 with Assay Buffer. i.e take 40 µl PAF-AH Enzyme to 160 µl Assay Buffer, Mix well. Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl PAF-AH Enzyme Solution:

PAF-AH Assay Buffer	45 µl
Diluted PAF-AH Enzyme*	5 µl

   Mix and add 50 µl of the PAF-AH Enzyme Solution into the desired wells. ***Do not save the diluted PAF-AH Enzyme. Discard it after using.**

2. **Screen Compounds, Inhibitor Control, and Blank Control Preparation:** Dissolve candidate inhibitors into appropriate solvent at 100X the highest final concentration to be tested. Dilute to 4X the desired test concentration with PAF-AH Assay Buffer. Add 50 µl diluted test inhibitor, or PAF-AH Assay Buffer into wells containing PAF-AH Enzyme, as Sample Screen [S], or Enzyme Control [EC] (no inhibitor). Dilute Inhibitor (Methyl Arachidonyl Fluorophosphonate) by adding 5 µl of PAF-AH Inhibitor Control to 245 µl of PAF-AH Assay Buffer. Add 50 µl of diluted Inhibitor Control into desired well(s). Adjust the volume of Sample, Enzyme Control, and Inhibitor Control wells to 100 µl/well with PAF-AH Assay Buffer. Incubate at room temperature for 5 min.

   Note: Use diluted PAF-AH Inhibitor Control within 4 hrs.

3. PAF-AH Substrate Solution Preparation: For each well, prepare 50 µl of Substrate solution.

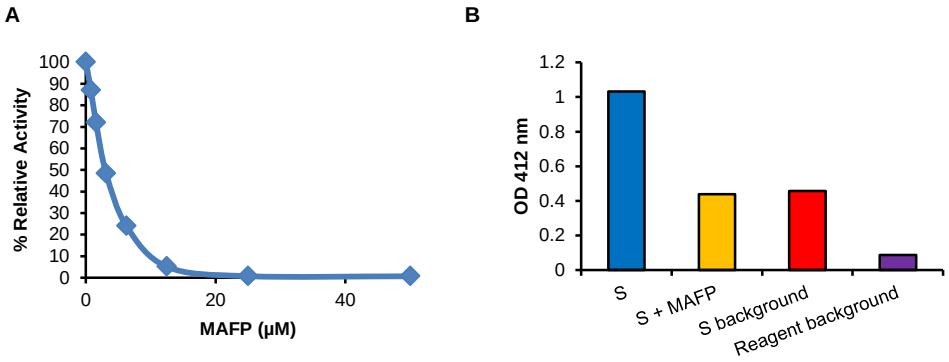
PAF-AH Substrate	2 µl
DTNB Solution	1 µl
PAF-AH Assay Buffer	47 µl

Mix and add 50 µl of Substrate solution into each well. Mix well. Incubate for more than 60 min at RT, protected from light.

4. Measurement: Measure absorbance at OD 412 nm.

5. Calculation: Set the absorbance of enzyme control (EC) as 100%, and calculate the relative % inhibition of test inhibitors as follows:

$$\% \textbf{ Inhibiton} = \frac{\textbf{OD 412 nm of EC} - \textbf{OD 412 nm of S}}{\textbf{OD 412 nm of EC}} \textbf{ X 100}$$



Figures: A). Inhibition of PAF-AH activity by PAF-AH Inhibitor (MAFP, Methyl Arachidonyl Fluorophosphonate), IC_{50} = 3 µM. **B).** PAF-AH activity was measured in perfused rat kidney in the presence and absence of 40 µM PAF-AH inhibitor (Methyl Arachidonyl Fluorophosphonate). S: 10 mg of rat kidney was homogenized in 100 µl of PAF-AH Assay Buffer, put into ice for 10 min, centrifuge at 10,000 g for 5 min, and collect the supernatant. Take 3 µL of rat kidney lysate for assay (70 µg). Assays were performed following the kit protocol.

VII. Related Products:

Myeloperoxidase (MPO) Colorimetric Assay Kit (K744)
PLTP Activity Assay Kit II (K593)
MPO Inhibitor Screening Kit (K746)
CEPT Activity Assay Kit II (K595)
Sphingomyelin Quantification Assay Kit (K600)
Sphingomyelinase Activity Fluorometric Assay Kit (K574)
CETP Blocking Peptide (3413BP)
Myeloperoxidase Fluorometric Assay Kit (K745)
Lipid Peroxidation (MDA) Assay Kit (K739)
MPO Peroxidation Assay Kit (K747)
Sphingomyelinase Activity Assay Kit (K599)
CETP Inhibitor Screening Kit II (K594)
CETP Antibody (3413)
Active Recombinant Human CETP (7606)

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