PAF Acetylhydrolase (PAF-AH) Inhibitor Screening Kit (Colorimetric)  
(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-acetylglycero-phosphocholine esterase or Lipoprotein-associated Phospholipase A2 or Lp-PLA2) (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.

II. Application:  
- Screening/studying/characterizing potential PAF-AH inhibitors

III. Kit Contents:

<table>
<thead>
<tr>
<th>Components</th>
<th>K766-100</th>
<th>Cap Color</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAF-AH Assay Buffer</td>
<td>50 ml</td>
<td>NM</td>
<td>K766-100-1</td>
</tr>
<tr>
<td>DTNB (in DMSO)</td>
<td>100 µl</td>
<td>Red</td>
<td>K766-100-2</td>
</tr>
<tr>
<td>PAF-AH Substrate (in EtOH)</td>
<td>100 µl</td>
<td>Blue</td>
<td>K766-100-3</td>
</tr>
<tr>
<td>PAF-AH Enzyme</td>
<td>100 µl</td>
<td>Purple</td>
<td>K766-100-4</td>
</tr>
<tr>
<td>Inhibitor (in DMSO)</td>
<td>40 µl</td>
<td>Black</td>
<td>K766-100-5</td>
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</table>

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 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.

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

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAF-AH Substrate</td>
<td>2 µl</td>
</tr>
<tr>
<td>DTNB Solution</td>
<td>1 µl</td>
</tr>
<tr>
<td>PAF-AH Assay Buffer</td>
<td>47 µl</td>
</tr>
</tbody>
</table>

Mix and add 50 µl of Substrate solution into each well. Mix well. Incubate for 40 min. at room temperature, protected from light.


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

\[
\% \text{ Inhibition} = \frac{\text{OD}_{412 \text{ nm of EC}} - \text{OD}_{412 \text{ nm of S}}}{\text{OD}_{412 \text{ nm of EC}}} \times 100
\]

Figures: A). Inhibition of PAF-AH activity by PAF-AH Inhibitor (MAFP, Methyl Arachidonyl Fluorophosphonate), IC\textsubscript{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)
- Myeloperoxidase Fluorometric Assay Kit (K745)
- PLTP Activity Assay Kit II (K933)
- Lipid Peroxidation (MDA) Assay Kit (K739)
- MPO Inhibitor Screening Kit (K746)
- MPO Peroxidation Assay Kit (K747)
- CEPT Activity Assay Kit II (K595)
- Sphingomyelinase Activity Assay Kit (K599)
- Sphingomyelin Quantification Assay Kit (K600)
- CETP Inhibitor Screening Kit II (K594)
- Sphingomyelinase Activity Fluorometric Assay Kit (K574)
- CETP Antibody (3413)
- CETP Blocking Peptide (3413BP)
- Active Recombinant Human CETP (7606)