PAF Acetylhydrolase Activity Assay Kit (Colorimetric)

(Catalog # BN00978; 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. Upregulated 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. Therefore early detection of PAF-AH activity is critical for mechanistic study, diagnosis, prevention, and therapeutic purpose. Assay Genie's PAF Acetylhydrolase Assay kit provides a quick and easy way for monitoring PAF-AH activity in a variety of samples. In this kit, PAF-AH hydrolyzes the acetyl thioester bond at sn-2 position of substrate and free thiols are detected using DTNB. The assay is simple, sensitive, and high-throughput adaptable. Detection limit: < 0.1mU.

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

II. Application:

- Measurement of PAF-AH activity in various samples
- Study/characterize PAF-AH inhibitors or activators
- Mechanistic study of inflammatory disorders

III. Sample Type:

- Animal tissues such as liver, kidney, etc.
- Adherent or suspension cells
- Serum or plasma

IV. Kit Contents:

Components	BN00978	Cap Code	Part Number
PAF-AH Assay Buffer	50 ml	NM	BN00978-1
PAF-AH Substrate	100 µl	Blue	BN00978-2
DTNB Probe (in DMSO)	100 µl	Red	BN00978-3
TCEP	50 µl	Clear	BN00978-4
PAF-AH Positive Control	Vial	Orange	BN00978-5

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

VI. Storage Conditions and Reagent Preparation:

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

- **PAF-AH Assay Buffer:** Warm to room temperature before use. Store at -20°C or 4°C.
- **PAF-AH Substrate:** Evaporate ethanol from PAF-AH Substrate vial (e.g. use 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.
- **DTNB Probe:** Before use, thaw at room temperature. Store at -20°C.
- **PAF-AH Positive Control:** Reconstitute with 80 µl PAF-AH Assay Buffer and mix thoroughly. Aliquot and store at –70°C. Keep on ice while in use. Use within two months.

VII. PAF-AH Activity Assay Protocol:

1. **Sample Preparation: Extracellular PAF-AH:** Serum or plasma samples can be measured directly. Add 10-50 µl sample per well and adjust the volume to 100 µl/well with PAF-AH Assay Buffer.

   Intracellular PAF-AH: Rapidly homogenize tissue (5 mg) or cells (1 x 10^6) with 100 µl ice cold PAF-AH Assay Buffer. Keep on ice for 10 min. Centrifuge at 10,000 X g for 5 min. and collect supernatant. Add 10-50 µl supernatant per well and adjust the volume to 100 µl/well with PAF-AH Assay Buffer.

   For PAF-AH Positive Control, take 2-20 µl of PAF-AH Positive Control into desired well(s) and adjust the volume to 100 µl/well with PAF-AH Assay Buffer.

   Notes:

   a. For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

   b. For samples having background such as GSH etc., prepare parallel sample well(s) as sample background control(s).

2. **Standard Curve:** To prepare TNB Standard, add 2.5 µl DTNB Probe and 2.5 µl TCEP into 245 µl of PAF-AH Assay Buffer and mix well. Make as much as needed. Add 0, 10, 20, 30, 40 and 50 µl of TNB Standard into a series of wells in a 96-well plate to generate 0, 5, 10, 15, 20 and 25 nmol/well of TNB Standard. Adjust the volume to 200 µl/well with PAF-AH Assay Buffer.

Notes:

a. Prepare TNB Standard just before use as it is easily oxidized. Discard unused Standard. TNB ε = 13600 m^{-1} cm^{-1}

b. Since 1 part of DTNB generates 2 parts of TNB, the TNB Standard has been adjusted by factor 2.

2. **Reaction Mix:** Mix enough reagents for the number of assays to be performed.

Extracellular PAF-AH: For each well, prepare 98 µl Reaction Mix containing:

	Reaction Mix	Background Control Mix*
PAF-AH Assay Buffer	97 µl	99 µl
DTNB Probe	1 µl	1 µl

Mix well by vortexing. Add 98 µl of Reaction Mix to each well containing Positive Control and samples. Mix and incubate at room temperature for 30 min. After incubation, add 2 µl of PAF-AH Substrate into Positive Control and sample wells. Mix well.

*Add 100 µl of Background Control Mix to sample background control well(s).

Intracellular PAF-AH: For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix	Background Control Mix
PAF-AH Assay Buffer	48 µl	50 µl
PAF-AH Substrate	2 µl	----

Mix well and add 50 µl of Reaction Mix to each well containing Positive Control and samples. Incubate at room temperature for 30 min. Prepare mix of 1 µl of DTNB and 49 µl of PAF-AH Assay Buffer for each well. Mix well by vortexing. Make as much as needed, add 50 µl of this mix to Positive Control, background control, and sample wells. Mix well.

4. **Measurement:**

Extracellular PAF-AH: Measure absorbance (412 nm) immediately in kinetic mode for 20-60 min. at room temperature.

Note: Incubation time depends on the PAF-AH activity in the samples. We recommend measuring OD in kinetic mode, and choosing two time points (T_1 & T_2) in the linear range to calculate the PAF-AH activity of the samples. The TNB Standard Curve can be read in Endpoint mode (i.e., at the end of incubation time).

Intracellular PAF-AH: Measure absorbance (412 nm) immediately at room temperature (End-point).

5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the TNB Standard Curve. If sample background control reading is significant, subtract sample background control reading from sample reading.

Extracellular PAF-AH: Calculate the PAF-AH activity of the test sample: $\Delta OD = A_2 - A_1$. Apply ΔOD to the TNB Standard Curve to get B nmol of TNB generated by PAF-AH during the reaction time ($\Delta T = T_2 - T_1$).

Intracellular PAF-AH: Apply corrected OD to the TNB Standard Curve to get B nmol of TNB generated by PAF-AH during the incubation time (T = 30 min).

Sample PAF-AH Activity = B/(T* X V) x Dilution Factor = nmol/min/µl = mU/µl = U/ml

Where: **B** is TNB amount in the sample well from Standard Curve (nmol).

T* is reaction time (min.). For extracellular PAF-AH activity, it is $\Delta T = T_2 - T_1$ and for intracellular PAF-AH activity, T = 30 min.

V is sample volume added into the reaction well (µl).

Unit Definition: One unit of PAF-AH is the amount of enzyme that generates 1.0 µmol of TNB per min. at pH7.2 at 25°C.

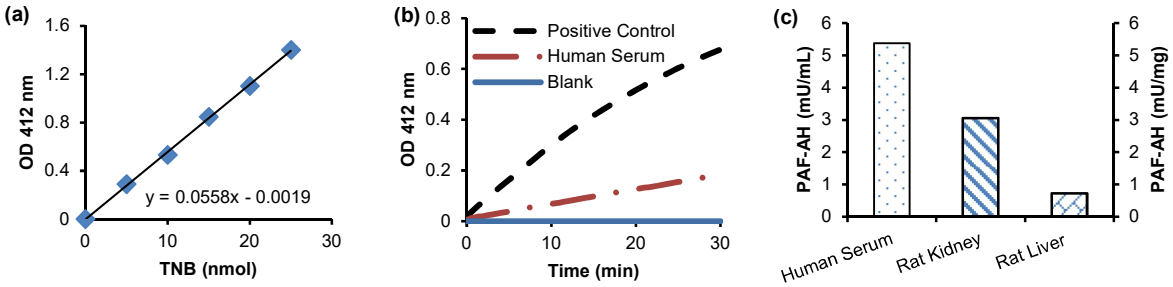

Figure: (a) TNB Standard Curve. (b) Measurement of PAF-AH activity in human serum. (c) Relative PAF-AH Activity was calculated in human serum (20 µl) and lysates prepared from rat kidney (75 µg) and liver (45 µg). Assay was performed following the kit protocol.

FOR RESEARCH USE ONLY! Not to be used on humans.