ab287879 – PAF Acetylhydrolase Activity Assay Kit

A simple, sensitive, and high-throughput adaptable assay for the measurement of PAF-AH activity in serum, plasma, animal tissues, and adherent or suspension cells.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
https://www.abcam.com/ab287879

Storage and Stability

Store kit at -20°C, protected from light.

Materials Supplied

Item	Quantity	Storage Condition
DTNB Probe (in DMSO)	100 µl	-20°C
PAF-AH Assay buffer	50 ml	-20°C
PAF-AH Positive Control	Vial	-20°C
PAF-AH Substrate	100 µl	-20°C
TCEP	50 µl	-20°C

Materials Required, Not Supplied

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

- Multi-well spectrophotometer (ELISA reader)

Reagent Preparation

- Briefly centrifuge small vials prior to opening.

PAF-AH Assay Buffer: Warm to room temperature before use.

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.

DTNB Probe: Before use, thaw at room temperature.

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.

Standard Preparation

- 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.
- Prepare TNB Standard just before use as it is easily oxidized. Discard unused Standard. TNB ε = 13600 m-1 cm-1.
- Since 1 part of DTNB generates 2 parts of TNB, the TNB Standard has been adjusted by factor 2.

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 106) 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.
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.

Δ Note: *For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range. For samples having background such as GSH etc., prepare parallel sample well(s) as sample background control(s).*

Assay Protocol

- Prepare TNB Standard just before use as it is easily oxidized. Discard unused Standard. TNB ε = 13600 m-1 cm-1
- Since 1 part of DTNB generates 2 parts of TNB, the TNB Standard has been adjusted by factor 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:

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

1. Mix well. 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.
2. *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:

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

1. Mix well and add 50 µl of Reaction Mix to each well containing Positive Control and samples. Incubate at room temperature for 30 min.
2. 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.

Measurement

Extracellular PAF-AH: Measure absorbance (412 nm) immediately in kinetic mode for 20-60 min. at room temperature.
Δ Note: *Incubation time depends on the PAF-AH activity in the samples. We recommend measuring OD in kinetic mode and choosing two time points (T1 & T2) 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).

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: ΔOD = A2 – A1. Apply ΔOD to the TNB Standard Curve to get B nmol of TNB generated by PAF-AH during the reaction time (ΔT = T2 - T1).

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** = TNB amount in the sample well from Standard Curve (nmol).
T* = Reaction time (min.). For extracellular PAF-AH activity, it is ΔT = T2 - T1 and for intracellular PAF-AH activity, T = 30 min.
V = 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.

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
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