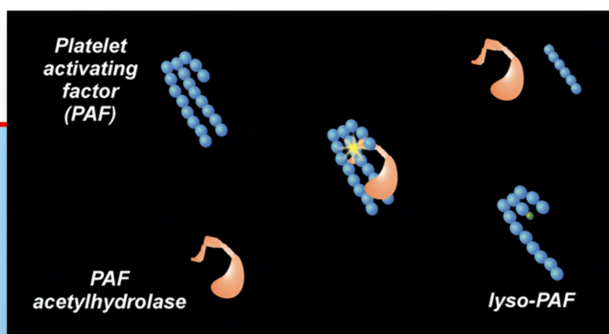

Auto PAF-AH

Serum (plasma) platelet-activating factor (PAF) acetylhydrolase assay

Instruction Manual

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Research Product

AZWELL Auto PAF-AH

Serum (plasma) platelet-activating factor (PAF) acetylhydrolase assay

Platelet-activating factor (PAF) is a biologically active phospholipid synthesized by a variety of mammalian cell types. It is a mediator for allergic and inflammatory reactions. PAF is converted to the biologically inactive lyso-PAF by PAF acetylhydrolase (PAF-AH), which catalyzes the hydrolysis of the esterified acetate at the *sn*-2 position. PAF-AH also degrades oxidized phospholipids that are structurally similar to PAF and closely associated with atherosclerosis. These observations suggest that PAF-AH plays an important role in allergic, inflammatory, and atherosclerotic diseases. Changes in human plasma or serum PAF-AH activity have been observed in a variety of diseases. **AZWELL Auto PAF-AH** is an accurate and convenient assay for the measurement of serum (plasma) PAF-AH activity [ref 1].

[Reagents]

R1: 200 mmol/L HEPES buffer, pH 7.6.
R2A: 20 mmol/L citric acid monohydrate buffer, pH 4.5.
R2B: 1-myristoyl-2-(4-nitrophenylsuccinyl)phosphatidylcholine, 90 mmol/L.

[Application]

For the measurement of PAF-AH activity in serum or plasma

[Assay Method]

1. Method
   Spectrophotometry
2. Principles [ref 1]
   PAF-AH hydrolyzes the *sn*-2 position of the substrate (1-myristoyl-2-(4-nitrophenylsuccinyl) phosphatidylcholine), producing 4-nitrophenyl succinate. This compound immediately degrades in aqueous solution and liberates 4-nitrophenol. This liberation is monitored spectrophotometrically and the activity is measured by the change in absorption.
3. Features
   (1) It does not require any pretreatment of samples.
   (2) It does not require radioisotopes.
   (3) It is applicable to an automatic analyzer.
   (4) It is a liquid product (not a lyophilized product), and is stable for 11 months at 2–8 °C
   (5) It lasts for 14 days after preparation of the R2 solution.
   (6) The assay is linear up to 1500 IU/L.
   (7) It is unaffected by a variety of esterases.
   (8) It is unaffected by hemoglobin, bilirubin, or chylomicron.

Using this assay with an automatic analyzer, it is possible to measure the activities of thousands of samples in a few hours.

[Preparation and Procedure]

1. Reagent preparation
   R1: Use buffer solution as supplied. Store at 2–8 °C.
   R2: Mix R2A and R2B in the proportion of 19:1 before use (for example: R2A, 19 mL + R2B, 1 mL). Store at 2–8 °C.
   CAUTION! <u>**Once R2A and R2B are mixed, the mixture (R2) must be used within two weeks. Do not keep it for longer.**</u>
2. Test procedure
   AZWELL Auto PAF-AH is intended for use as a reagent for the measurement of serum (plasma) PAF-AH activity based on the above-mentioned principle. This reagent can be used with various automatic analyzers. The following example is for Hitachi model 7170 automatic analyzer (Hitachi Ltd., Tokyo, Japan). Please read and follow the instrument manufacturer's instruction manual when using the kit.

Procedure 1 (for automatic analyzer)

(1) After entering the parameters from the following table into the automatic analyzer program, the analytical procedure described below is performed automatically.

Test sample (2 μL) + R1 (240 μL) 37 °C, 5 min [0–5 min] Add R2 (80 μL) 37 °C, 5 min [5–10 min] Measure the Absorbance [6–8 min] Calculate PAF-AH activity

Absorbance: difference between 405 nm and 505 nm
Reagent blank: Purified water or physiological saline

(2) Parameters for Hitachi 917 automatic analyzer

ITEM	INPUT
TEST	[PAF-AH]
ASSAY CODE	[Rate-A] [10] [21] [28] [0] [0
WAVELENGTH (Sub/Main)	[505] [405]
SAMPLE VOLUME	[2.0]
R1 VOLUME	[240]
R3 VOLUME	[80]
Calibration	
CALIB. METHOD	[LINEAR]
Standard	
STD.(1)CONC.–POS.–VOL.	[0] [water(99)] [2.0]
K-factor	
Determine your own K-factor	

Procedure 2

(1) Aliquots of 2 μL of sample and 240 μL of R1 are mixed, and pre-incubated at 37 °C for 5 min. The reaction is then started by adding 80 μL of R2. The absorption is measured at 405 and 505 nm (main and sub wavelength, respectively) at 1 and 3 min after the addition of R2. An aliquot of 2 μL of water is also used as a blank control.

(2) Changes in the absorbance per minute are used for

calculation of the activity, using the difference between the change in absorbance of the sample (EA), the change in absorbance of the blank (EB), and the extinction coefficient of 4-nitrophenol.

(3) Calculation of activity
PAF-AH activity (IU/L) = (EA– EB) × V × 10^6 / (× sv × d), where:
V: final volume of reaction mixture (μL)
10^6: transformation from moles to micromoles
 : the extinction coefficient of 4-nitrophenol
sv: sample volume (μL)
d: light path (cm)

[Sample]

1. Plasma or serum can be used as a sample. The sample should be assayed as soon as possible after blood collection.
2. Keep samples at 2–8 °C. Freeze the sample if you store for more than 24 hours. Do not repeatedly freeze-and-thaw.

[Performance]

1. Sensitivity
   (1) Reagent blank: less than 0.01 abs/min
   (2) Sensitivity: 0.02–0.06 abs/min at an activity of 500 IU/L
2. Specificity
   90–110% of expected assay value
3. Reproducibility
   Coefficient of variation: less than 5%
4. The following potentially interfering substances hardly affect the assay values. [ref 2]
   (1) Hemoglobin, up to 500 mg/100 mL
   (2) Glucose, up to 1000 mg/100 mL
   (3) Uric acid, up to 50 mg/100 mL
   (4) Urea, up to 200 mg/100 mL
   (5) Conjugated bilirubin, up to 20 mg/100 mL
   (6) Free bilirubin, up to 20 mg/100 mL
   (7) Ascorbic acid, up to 50 mg/100 mL

[Measurement range] [ref 2]

10–1500 IU/L

[Correlation] [ref 1]

Sample: human serum
N = 100
R = 0.979
$y = 15.877x + 14.884$
X-axis: the value by radioisotopic assay
Y-axis: the value by Azwell Auto PAF-AH

[Reference normal value in Japanese]

Less than 800 IU/L [ref 2]

[Precautions]

1. Avoid exposing the reagents to direct sunlight. Store the reagents at 2–8 °C. Avoid freezing. Do not use any reagents that are expired. Do not mix any reagents with differing lot numbers.
2. Once the R2 (R2A-R2B mixture) is prepared, it will last no longer than 14 days.
3. Samples should be handled with care to avoic infection, as they may contain infectious agents suc as HBV, HCV, and HIV.
4. Dilute the sample with saline or water and measure its activity again if the sample activity exceeded the measurement range.
5. Clotting may clog up a sample probe. Remove any visible clots before testing.
6. Bubbles in the sample may affect the value, so avoic frothing.
7. Do not re-use the reagent bottles, and do not use the preparations, test solutions and reagents for any purpose other than described herein.
8. R2A or R2 solutions are pH 4.5. Please flush with ε large amount of water when discarding, or discarc them according to your facility's own rule or relatec law.
9. The solvent involved in R2B is flammable liquic (alcohol). Avoid using it near fire.
10. Avoid contact with eyes. If this occurs, immediately flush eyes with plenty of water and then go to the doctors.

[Storage]

2–8 °C

[Shelf life]

Eleven months after production. (Expiry date is indicated on the package.)

[Supplied volume]

R1: 60 mL × 2
R2A: 19 mL × 2
R2B: 2.2 mL × 1

[Note]

For research use only. Do not use in diagnostic or therapeutic procedures.

[References]

[1] Kosaka T., Yamaguchi M., Soda Y., Kishimoto T. Tago A., Toyosato M., and Mizuno K. (2000) A spectrophotometric assay for serum platelet-activating factor acetylhydrolase activity. Clinicε Chimica Acta 296, 151-161.
[2] In-house data of Azwell, Inc.

[K-factor]

We recommend that you determine your own K-factor or (molar extinction coefficient) with your automatic analyzer or spectrophotometer, 4-nitrophenol, and the reagents R1 and R2A, without R2B substrate, to estimate PAF-AH activity precisely.

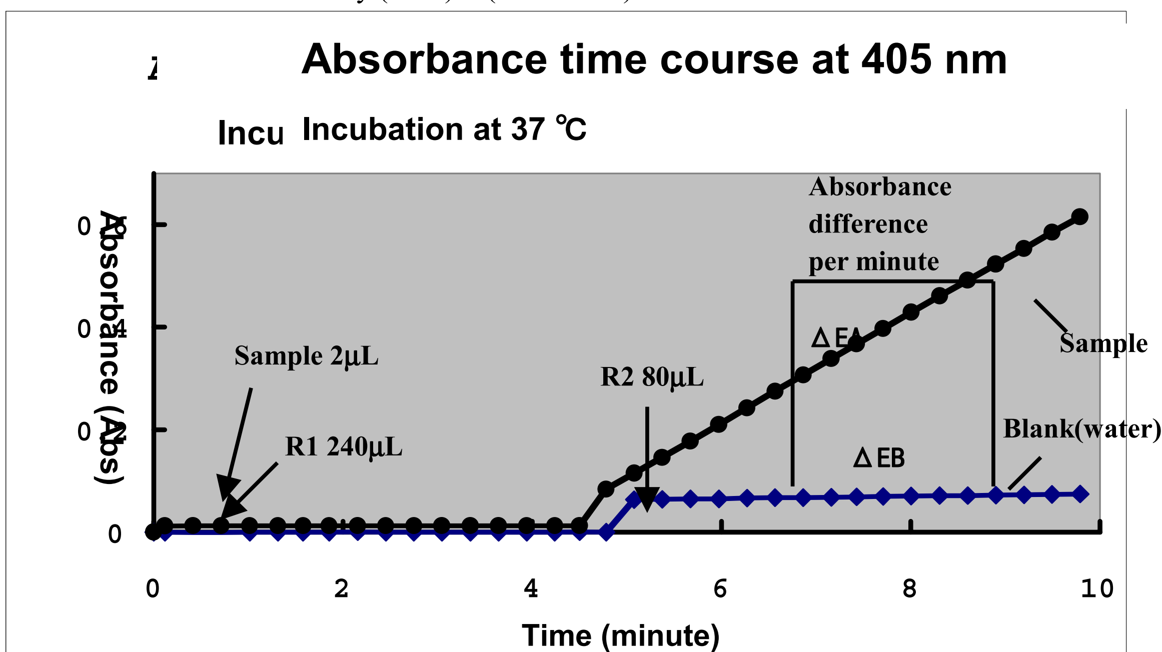
PROTOCOL FOR A STANDARD SPECTROPHOTOMETER

Please refer to the following procedure if you perform the PAF-AH assay using a standard spectrophotometer.

PROCEDURE

(1) Aliquots of 2 μL of sample and 240 μL of R1 are mixed, and pre-incubated at 37 °C for 5 min. The reaction is then started by adding 80 μL of R2. The absorption is measured at 405 nm at 1 and 3 min after the addition of R2. An aliquot of 2 μL of water is also used as a blank control.

(2) Changes in the absorbance per minute are used for calculation of the activity, using the difference between the change in absorbance of the sample (ΔEA), the change in absorbance of the blank (ΔEB), and K-factor. PAF-AH activity (IU/L) = (ΔEA–ΔEB) × K



Please determine your own K-factor. You could prepare your own 4-nitrophenol solution precisely, however, you could also use the following commercially available kit, which is designed for setting K-factor.
WAKO Chemicals, 433-97501, K-factor Calibrator 4-Nitrophenol (5mL x 4).
Available from: Wako Chemicals USA, Inc. (www.wakousa.com)

HOW TO CALCULATE K-FACTOR

(1) Determine the concentrations of 4-nitrophenol solutions. (Cs1, Cs2, and Cs3 in the following table)

(2) Do your own assay using a blank solution and 4-nitrophenol solutions as a sample, and determine each absorbances. (n=5; Ab, Ab1, Ab2, and Ab3 in the following table) (In this assay, you must use R2A instead of R2. However, use R2 in the assay when you estimate your sample. R2 means R2A-R2B mix in the proportion of 19:1.)

(3) Calculate the mean values of Ab, Ab1, Ab2, and Ab3.

(4) Blank corrections (-Blank): Subtract the mean value of Ab from mean values of Ab1, Ab2, Ab3, respectively.

(5) Calculate the relative absorbances (after blank correction) of Cs2 and Cs3 where the Cs1 (after blank correction) is regarded as 100. Use following equation.
Relative Abs of Cs2 (or Cs3) = [Cs1/Cs2(or Cs3)] X [Abs of Cs2(or Cs3)/Abs of Cs1] X 100

(6) Calculate K-factor of Cs1, Cs2, and Cs3
Ex. K-factor of Cs1 = [Cs1 conc (mmol/L)/Mean Abs of Cs1 after blank correction] X 10^7

(7) Determine the K-factor (average of the above K-factors)
Calculate the mean K-factors for Cs1, Cs2, and Cs3, and use it as the final K-factor. However, if any Relative Abs calculated in step 6 is not within the 100.0 +/- 3.0 %, eliminate such K-factor from the averaging calculation to obtain final K-factor.

	Blank	Cs1	Cs2	Cs3
Concentrations (mmol/L)	0.00			
	Ab	Ab1	Ab2	Ab3
Absorbances				
Means				
Blank corrections				
Relative Abs		100%	%	%
K-factors				

Ordinarily, the K-factor must be 11,000-13,000 in this way. (Light path = 1cm, sample:R1:R2 volumes ratio = 2:240:80)

Example

	Blank	Cs1	Cs2	Cs3
Concentrations (mmol/L)	0.00	1.01	2.02	3.02
	Ab	Ab1	Ab2	Ab3
Absorbances	0.0018	0.0846	0.1678	0.2523
	0.0016	0.0884	0.1666	0.2504
	0.0016	0.0862	0.1703	0.2560
	0.0017	0.0867	0.1661	0.2519
	0.0019	0.0840	0.1665	0.2524
Means	0.00172	0.08598	0.16746	0.2526
Blank corrections		0.08426	0.16574	0.25088
Relative Abs		100%	98.4%	99.6%
K-factors		11987	12188	12038

K = (11987+12188+12038)/3 = 12071