sPLA2 activity Kit

Catalog Number KA1015

96 assays

Version: 03

Intended for research use only

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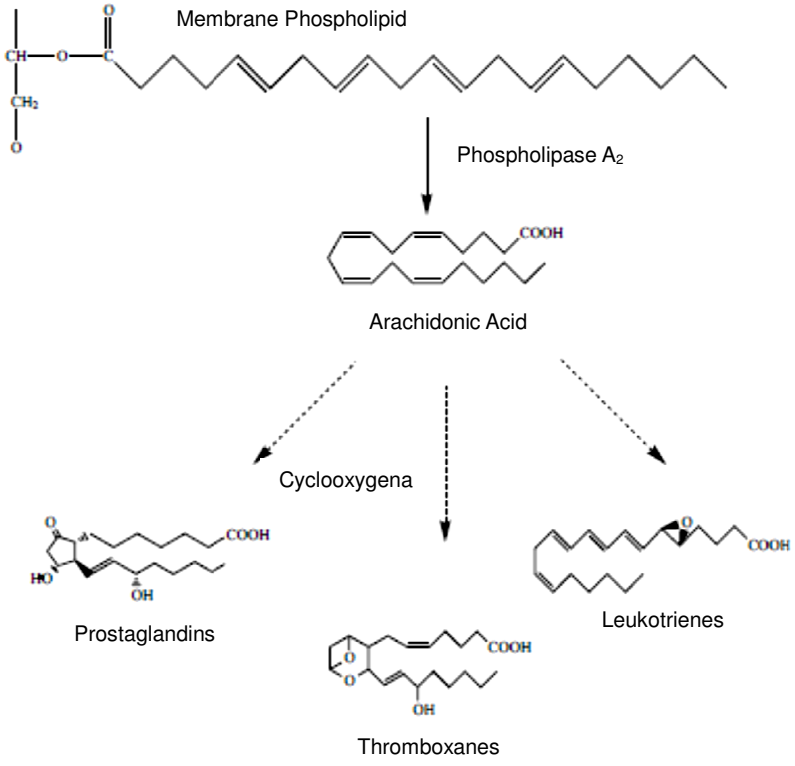
Introduction

Background

Secretory Phospholipase A_2 ($sPLA_2$) are a group of related enzymes that upon activation, cleave an acyl ester bond in the sn-2 position in glycerophospholipid.[1,2] These are extracellular enzymes that have high disulfide bond content, low molecular mass (14KDa), and require mM levels of Ca^+ for catalysis.[3] More importantly, these enzymes play a crucial role in the generation of arachidonates and the very powerful eicosanoids, proinflammatory mediators (figure 1).[4,5,6] PLA_2 has been shown to have a number of biological actions including immunological responses, inflammation, cellular proliferation, vasoconstriction, and bronchioconstriction.[1] The general scheme for the action of PLA_2 on arachidonic acid is given below.

Figure 1. The PLA_2 Reaction



Principle of the Assay

The $sPLA_2$ activity Kit is a complete kit for the quantitative determination of secretory Phospholipase A in biological fluids. Please read the complete kit insert before performing this assay. The kit uses a specific substrate for $sPLA_2$ that is converted into a sulfhydryl molecule. The presence of the sulfhydryl product is detected colorimetrically using Ellman's reagent, DTNB, which forms a yellow colored product with the sulfhydryl formed. The amount of $sPLA_2$ in the sample is compared to the amount of standard $sPLA_2$ supplied in the kit by comparison of the yellow color generated at 405 nm.

General Information

Materials Supplied

Component	Description	Amount
Microtiter Plate	A plate using break-apart strips. The plate is ready to use.	1 each
$sPLA_2$ Standard	Two vials of lyophilized standard at 40 units per vial. One unit will hydrolyze 1.0 µmole of soybean L-phosphatidylcholine to L-alpha lysophosphatidylcholine and fatty acid per minute at pH 8.0 at 37^0C.	2 vials
$sPLA_2$ Reaction Buffer Concentrate	A Tris based buffer containing sodium azide as preservative.	15 mL
$sPLA_2$ Substrate,	2 Vials of lyophilized HEPC	2 vials
Stop Reagent,	A solution of EDTA in buffer containing sodium azide as preservative	3 mL
$sPLA_2$ Color Reagent,,	A solution of DTNB in ethanol	3 mL
Plate Sealer	-	1 each

Storage Instruction

All components of this kit, except the sPLA2 Substrate, are stable at 4℃ until the kit's expiration date. The $sPLA_2$ Substrate must be stored at -20℃.

Materials Required but Not Supplied

- ✓ Deionized or distilled water.
- ✓ Precision pipets for volumes between 25 µL and 1,000 µL.
- ✓ Repeater pipets for dispensing 25, 50 and 100 µL.
- ✓ Disposable beaker and graduated cylinder for diluting buffer concentrates.
- ✓ A 37℃ Incubator.
- ✓ Microplate reader capable of reading at 405 nm, preferably with correction between 570 and 590 nm.
- ✓ Software for extrapolating sample values from optical density readings utilizing a four parameter logistic curve fit.

Precautions for Use

✓ Precautions

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Some kit components contain azide, which may react with lead or copper plumbing. When disposing of reagents always flush with large volumes of water to prevent azide build-up.
- The $sPLA_2$ Substrate, HEPC, is a lipid of unknown biological effects. Care should be used in handling this reagent.
- The Color Reagent is a solution of Ellman's Reagent, DTNB. Care should be used when handling this reagent.
- Do not mix components from different lots of kits.

✓ *Procedural Notes:*

- *Do not mix reagents from different kit lots or use reagents beyond the expiration date.*
- *Allow all reagents to warm to room temperature for at least 30 minutes before opening.*
- *Standards can be made up in either glass or plastic tubes.*
- *Mix the substrate well prior to use.*
- *Pre-rinse the pipet tip with the reagent and use fresh tips for each sample, standard and reagent.*
- *Add the reagents to the side of the well to avoid contamination.*
- *This kit uses break-apart microtiter plates, which allow the user to measure as many samples as desire. The wells should be used in the frame provided.*
- *The physical, chemical, and toxicological properties of the chemicals and reagents contained in this kit may not have been fully investigated. Therefore, we recommend the use of gloves, lab coats, and eye protection while using any of these chemical reagents.*
- *Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.*
- *The standard should be handled with care due to the known and unknown effects of the antigen.*
- *Bring all reagents to room temperature for at least 30 minutes prior to opening.*

Assay Protocol

Reagent Preparation

✓ Reaction Buffer, 1X

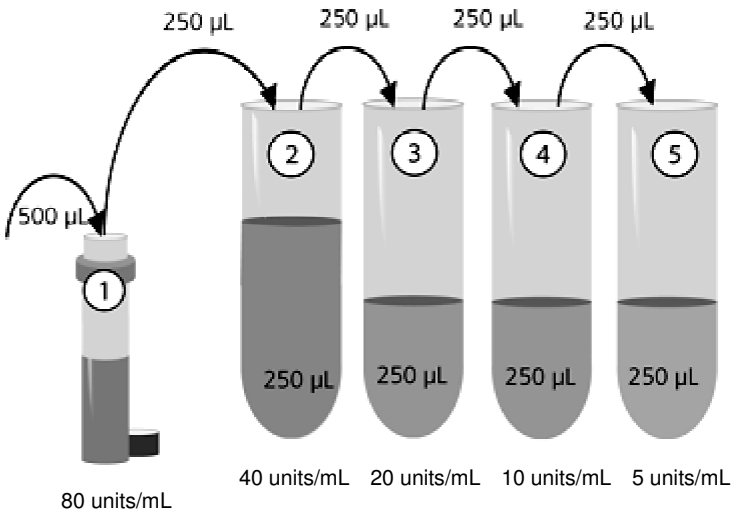
Prepare the 1X Reaction Buffer by diluting the supplied concentrate 1:5 with de-ionized water (for example: for an end volume of 25mLs of 1X reaction buffer, mix 5mLs of reaction buffer concentrate with 20mLs of diH_2O). Discard unused Reaction Buffer.

✓ $sPLA_2$ Substrate

Reconstitute a Substrate vial by adding 3 mL of deionized water. Wait for 5 minutes and vortex gently. After reconstitution, reagent is stable for 3 weeks at 4℃.

✓ $sPLA_2$ Standard

Reconstitute the 40unit lyophilized standard with 0.500mL 1x Reaction Buffer to achieve a working concentration of 80 units/mL and gently vortex. Wait 5 minutes and vortex again.
This solution contains 80units/mL and is tube #1. Label four 12 x 75 mm glass or plastic tubes #2 through #5. Pipet 250 µL of 1x Reaction Buffer into tubes #2 through #5. Pipet 250 µL of tube #1 into tube #2 and vortex thoroughly. Add 250 µL of tube #2 to tube #3 and vortex thoroughly.
Continue this for tubes #4 and #5.



Diluted standards should be used within 60 minutes of preparation. The concentrations of sPLA in the tubes are labeled above.

Sample Preparation

The $sPLA_2$ assay is compatible with $sPLA_2$ samples in a wide range of matrixes. Samples diluted into Reaction Buffer can be read directly from the standard curve. The activity of $sPLA_2$ enzymes is modulated by calcium ions. Samples should therefore be in 100mM Tris pH8.0, a buffer compatible with millimolar levels of calcium.

Because of the high affinity of calcium with phosphate ions, phosphate buffers cannot be used to measure $sPLA_2$ sample activity.

Note: If buffers other than those provided are used in the assay, the end-user must determine the appropriate dilution and assay validation.

Assay Procedure

Bring all reagents to room temperature for at least 30 minutes prior to opening.

All standards and samples should be run in duplicate.

1. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells back into the bag and seal the ziploc.
2. Pipet 50 µL of Standards #1 through #5 into duplicate wells.
3. Pipet 50 µL of Samples into duplicate wells.
4. Pipet 50 µL of Reaction Buffer into duplicate wells to act as a Blank (0 Standard).
5. Pipet 100 µL of Reaction Buffer into all wells.
6. Pipet 50 µL of the Substrate solution into all wells.
7. Mix well by shaking or tapping the side of the plate. Seal the plate with the plate sealer provided.
8. Incubate at 37℃ for 30 minutes.
9. Pipet 25 µL of the Stop Reagent into all wells.
10. Pipet 25 µL of the Color Reagent into all wells.
11. Mix well by shaking or tapping the side of the plate.
12. Incubate the plate at room temperature for 10 minutes.
13. Blank the plate reader against the Blank wells, read the optical density at 405nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the blank wells from all readings.

Note: All standards and samples should be run in duplicate.

Note: Pipet the reagents to the sides of the wells to avoid possible contamination.

Data Analysis

Calculation of Results

Several options are available for the calculation of the concentration of $sPLA_2$ in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic (4PL) curve fitting program.

Note: Make sure to multiply sample concentrations by the dilution factor used during sample preparation.

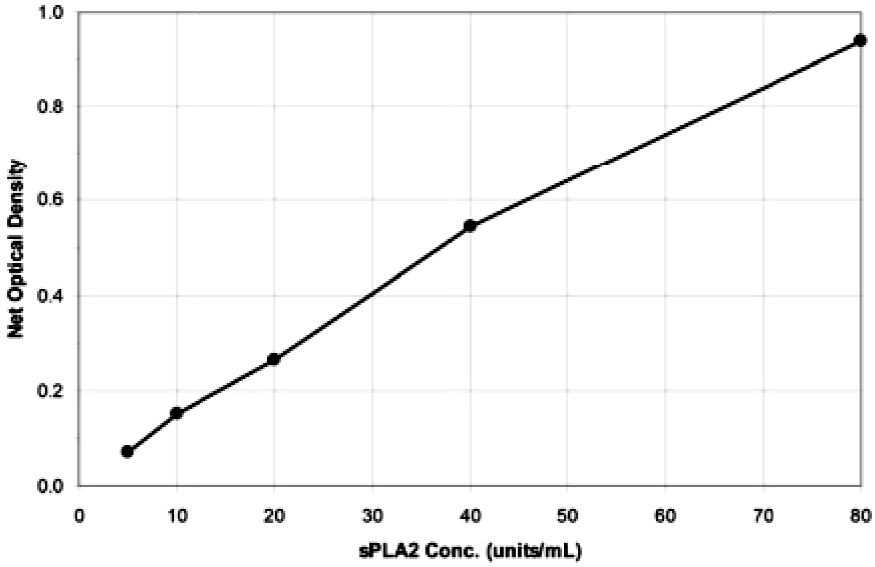
✓ Typical Results

The results shown below are for illustration only and should not be used to calculate results.

Sample	Average OD	Net OD	sPLA (units/mL)
Blank (0 Std.)	0.330		
S1	1.269	0.939	80
S2	0.872	0.542	40
S3	0.595	0.265	20
S4	0.481	0.151	10
S5	0.401	0.071	5

✓ Typical Standard Curve

A typical standard curve is shown below. The curve must not be used to calculate $sPLA_2$ concentrations; each user must run a standard curve for each assay.



Performance Characteristics

The following parameters for this kit were determined using the guidelines listed in the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols[9].

✓ Sensitivity

Sensitivity was calculated by determining the average optical density for twenty (20) wells run as blanks, and comparing to the average optical density for twenty (20) wells run with the low standard (Standard #5). The de tection limit was determined as the concentration of $sPLA_2$
measured at two (2) standard deviations below the Blank as measured by the standard curve.
The sensitivity of the assay was determined to be 2.34 units/mL.

✓ Precision

Intra-assay precision was determined by assaying 20 replicates of three buffer controls containing $sPLA_2$ in a single assay.

Units/mL	%CV
57.4	2.7
22.3	2.8
12.9	5.7

Inter-assay precision was determined by measuring buffer controls of varying $sPLA_2$ concentration in multiple assays over several days.

Units/mL	%CV
58.3	13.1
21.6	8.3
13.2	9.1

Resources

References

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2. O. Ohara, J. Ishizaki and H. Arita., Prog. Lipid Res.,(1995), 34 (2), 117-138.
3. E.A. Dennis, E.J. Ackerman, R.A. Deems, & L.J. Reynolds., in “Advances in Prostagladin, Thromboxane, and Leukotriene Research.”, (1995), 23, 75-79., Raven Press, NY, B.Samuelson, (Ed.).
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9. National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, 1989, NCCLS, Villanova, PA, 19085.

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Std4										
B	Blank	Std4										
C	Std1	Std5										
D	Std1	Std5										
E	Std2											
F	Std2											
G	Std3											
H	Std3											