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$sPLA_2$ (Type IIA) Inhibitor Screening Assay Kit

Item No. 702470

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -80°C kit. After opening the kit, store individual components as stated below.

Item Number	Item	Quantity/Size	Storage
400587	$sPLA_2$ Assay Buffer (5X)	1 vial/12 ml	-20°C
400595	$sPLA_2$ (Type IIA) Substrate Resuspension Buffer	1 vial/6 ml	-20°C
400603	$sPLA_2$ (Type IIA) Substrate	1 vial/600 µl	-20°C
400590	$sPLA_2$ Assay Probe	1 vial/6 ml	-20°C
400596	$sPLA_2$ (Type IIA) Enzyme	1 vial/200 µl	-80°C
400592	$sPLA_2$ Control Inhibitor	1 vial/200 µl	-20°C
400765	384-Well Clear Plate	1 plate	RT
400012	96-Well Cover Sheet	1 ea	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

This kit may not perform as described if any reagent or procedure is replaced or modified.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader with the ability to measure absorbance at a wavelength of 410 nm
2. Adjustable pipettes; multichannel or repeating pipettor recommended
3. A source of pure water; glass-distilled water or deionized water is acceptable
   NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000)

INTRODUCTION

Background

Secretory phospholipase A_2 ($sPLA_2$) (Type IIA) is a calcium-dependent PLA_2 superfamily member that is encoded by *PLA2G2A* in humans.[1] It is expressed in platelets, synovial fluid, vascular smooth muscle cells, adipose tissue, the liver, and the gut.[2,3] $sPLA_2$ (Type IIA) functions in a cell- and tissue type-specific manner, preferentially hydrolyzing bacterial membrane phosphatidylglycerol (PG) and phosphatidylethanolamine (PE) as a mechanism of host defense. It is upregulated at sites of acute and chronic inflammation where it generates lysophospholipids and free fatty acids, including arachidonic acid, that can be further metabolized to inflammatory lipid mediators and a variety of eicosanoid autocrine and paracrine signaling molecules.[4] Plasma levels of $sPLA_2$ (Type IIA) are associated with the presence of sepsis and are increased in patients with rheumatoid arthritis, acute coronary syndrome, asthma, or inflammatory bowel disease (IBD). Elevated plasma levels of $sPLA_2$ (Type IIA) are also associated with poor prognosis in patients with cancer and increased disease severity of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.[4,5] Based on these data, inhibition of $sPLA_2$ (Type IIA) is a potential therapeutic strategy in inflammatory diseases, including COVID-19 and cancer.[4-6]

About This Assay

Cayman's $sPLA_2$ (Type IIA) Inhibitor Screening Assay Kit provides a robust and easy-to-use platform for identifying novel inhibitors of human $sPLA_2$ type IIA, a phospholipase that preferentially hydrolyzes PG and PE. The assay uses diheptanoyl thio-PE, a sulfur-containing analog of PE as a substrate. Upon hydrolysis by $sPLA_2$ type IIA at the *sn*-2 position, the free thiol can be detected by the $sPLA_2$ Assay Probe. This results in a product that can be easily quantified by measuring absorbance at a wavelength of 410 nm. A potent and reversible inhibitor, LY315920 (included in this kit as $sPLA_2$ Control Inhibitor), inhibits the enzyme with an average IC_{50} value of approximately 22 nM.

PRE-ASSAY PREPARATION

Sample Preparation

All test compounds, be they small molecules, natural products, or proteins, should be prepared in a suitable solvent such as $sPLA_2$ Assay Buffer (1X), DMSO, dimethyl formamide (DMF), or short-chain alcohols (*e.g.*, MeOH, EtOH) at a concentration 35X the desired final assay concentration (*e.g.*, for 10 mM final assay concentration, a 350 mM stock should be made). The final concentration of organic solvents in the assay will then be ≤2.9% (see **Effects of Solvents** on page 18).

Reagent Preparation

1. **$sPLA_2$ Assay Buffer (5X) - (Item No. 400587)**

   Mix 10 ml of $sPLA_2$ Assay Buffer (5X) with 40 ml of pure water to make 50 ml of $sPLA_2$ Assay Buffer (1X). Once prepared, the $sPLA_2$ Assay Buffer (1X) may be stored at 4°C for at least three months.

2. **$sPLA_2$ (Type IIA) Substrate Resuspension Buffer - (Item No. 400595)**

   This vial contains 6 ml $sPLA_2$ (Type IIA) Substrate Resuspension Buffer. Once thawed, it is ready to use as supplied.

3. **$sPLA_2$ (Type IIA) Substrate - (Item No. 400603)**

   This vial contains 15.5 mg $sPLA_2$ (Type IIA) Substrate in ethanol. Completely evaporate all of the solvent under a gentle stream of inert gas (*e.g.*, nitrogen, argon). The dried substrate will appear as a clear, waxy solid. Add 4.7 ml of $sPLA_2$ (Type IIA) Substrate Resuspension Buffer to the vial and incubate in a 37°C water bath for 10 minutes. Vortex at full speed for one minute and then continue incubation in a 37°C water bath for an additional 10 minutes. The resuspended substrate will appear cloudy and will be stable at room temperature for at least three hours. If all of the resuspended substrate solution will not be used at one time, aliquot and store at 4°C where it will be stable for one month.

4. **$sPLA_2$ Assay Probe - (Item No. 400590)**

   This vial contains 6 ml of $sPLA_2$ Assay Probe. Once thawed, keep the probe on ice. It is ready to use as supplied. The $sPLA_2$ Assay Probe will be stable for up to four hours on ice. It can be aliquotted and stored at -20°C until the date of the expiration of this kit, limiting freeze-thaw cycles to three.

5. **$sPLA_2$ (Type IIA) Enzyme - (Item No. 400596)**

   $sPLA_2$ (Type IIA) Enzyme should be thawed on ice just prior to performing the assay and gently mixed, without vortexing. Use this enzyme to prepare the $sPLA_2$ (Type IIA) Master Mix as described on page 12. If all of the $sPLA_2$ (Type IIA) Enzyme will not be used at one time, aliquot it and store at -80°C. Avoid multiple freeze-thaw cycles.

6. **$sPLA_2$ Control Inhibitor - (Item No. 400592)**

   This vial contains 200 µl of 1 mM $sPLA_2$ Control Inhibitor in DMSO, which can be used as a positive control. It is ready to use as supplied. If all of the $sPLA_2$ Control Inhibitor will not be used at one time, aliquot it and store at -20°C.

ASSAY PROTOCOL

Plate Set Up

The 384-well plate(s) included with this kit is supplied ready to use. There is no specific pattern for using the wells on the plate. However, it is necessary to have four wells designated as vehicle and four wells designated as background. It is suggested that each potential inhibitor, including the positive control, be assayed in triplicate (quadruplicate preferred). A typical layout of samples to be measured in quadruplicate is shown in Figure 1, below.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	V	V	6	6	14	14	22	22	30	30	38	38	46	46	54	54	62	62	70	70	78	78	86	86
B	V	V	6	6	14	14	22	22	30	30	38	38	46	46	54	54	62	62	70	70	78	78	86	86
C	BW	BW	7	7	15	15	23	23	31	31	39	39	47	47	55	55	63	63	71	71	79	79	87	87
D	BW	BW	7	7	15	15	23	23	31	31	39	39	47	47	55	55	63	63	71	71	79	79	87	87
E	PC	PC	8	8	16	16	24	24	32	32	40	40	48	48	56	56	64	64	72	72	80	80	88	88
F	PC	PC	8	8	16	16	24	24	32	32	40	40	48	48	56	56	64	64	72	72	80	80	88	88
G	1	1	9	9	17	17	25	25	33	33	41	41	49	49	57	57	65	65	73	73	81	81	89	89
H	1	1	9	9	17	17	25	25	33	33	41	41	49	49	57	57	65	65	73	73	81	81	89	89
I	2	2	10	10	18	18	26	26	34	34	42	42	50	50	58	58	66	66	74	74	82	82	90	90
J	2	2	10	10	18	18	26	26	34	34	42	42	50	50	58	58	66	66	74	74	82	82	90	90
K	3	3	11	11	19	19	27	27	35	35	43	43	51	51	59	59	67	67	75	75	83	83	91	91
L	3	3	11	11	19	19	27	27	35	35	43	43	51	51	59	59	67	67	75	75	83	83	91	91
M	4	4	12	12	20	20	28	28	36	36	44	44	52	52	60	60	68	68	76	76	84	84	92	92
N	4	4	12	12	20	20	28	28	36	36	44	44	52	52	60	60	68	68	76	76	84	84	92	92
O	5	5	13	13	21	21	29	29	37	37	45	45	53	53	61	61	69	69	77	77	85	85	93	93
P	5	5	13	13	21	21	29	29	37	37	45	45	53	53	61	61	69	69	77	77	85	85	93	93

V = Vehicle Wells
BW = Background Wells
PC = Positive Control Wells
1-93 = Test Wells

Figure 1. Sample plate format

Pipetting Hints

- It is recommended that a multichannel pipette be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 70 µl in all the wells.
- All reagents should be prepared as described.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended that samples be assayed in quadruplicate.
- The assay is performed at room temperature.
- Monitor the absorbance at a wavelength of 410 nm.

Performing the Assay

NOTE: For best performance, the Master Mixes should be prepared just prior to performing the assay. If not using immediately, they should be kept on ice where they will be stable for up to 30 minutes. The assay may be read kinetically or as an endpoint. See steps 7 and 8 for details.

1. **Preparation of Master Mixes**: prepare a sufficient volume of the assay master mixes according to the table below. Scale the volumes up or down as needed. Each well requires 58 µl of the appropriate master mix.

	Background Master Mix (For Background Wells)	$sPLA_2$ (Type IIA) Master Mix (For Vehicle, Test Compound, and Positive Control Wells)
$sPLA_2$ Assay Buffer (1X)	240 µl	21.42 ml
$sPLA_2$ Assay Probe	50 µl	4,500 µl
$sPLA_2$ (Type IIA) Enzyme	--	180 µl
Final Volume (# of wells)	290 µl (5 wells)	26.1 ml (450 wells)

Table 1. Master mix preparation

2. **Background Wells**: add 58 µl of the Background Master Mix and 2 µl of solvent used to dissolve the test compounds/positive control to four wells. Mix gently by pipetting up and down 4-5 times being careful to avoid forming bubbles. If different solvents are to be assayed at the same time, separate sets of background wells should be run for each solvent.
3. **Vehicle Wells**: add 58 µl of the $sPLA_2$ (Type IIA) Master Mix and 2 µl of solvent used to dissolve the test compounds/positive control to four wells. Mix gently by pipetting up and down 4-5 times being careful to avoid forming bubbles. If potential inhibitors in different solvents are to be assayed at the same time, separate sets of vehicle wells should be run for each solvent.
4. **Test/Control Inhibitor Wells**: add 58 µl of the $sPLA_2$ (Type IIA) Master Mix to the test/control inhibitor wells on the plate. Add 2 µl of test compound or the $sPLA_2$ Control Inhibitor to four wells. Mix gently by pipetting up and down 4-5 times being careful to avoid forming bubbles.
5. Cover the plate with the 96-Well Cover Sheet (Item No. 400012) and incubate for 15 minutes at room temperature.
6. Initiate the reactions by adding 10 µl of resuspended $sPLA_2$ (Type IIA) Substrate to all the wells being used. Thorough mixing of the reaction solution is crucial for assay performance. Mix gently by pipetting up and down 4-5 times being careful to avoid forming bubbles. Pipetting a volume of 30-40 µl will ensure effective mixing.
7. If reading kinetically, skip this step. If reading as an endpoint, cover the plate with the 96-Well Cover Sheet and incubate for 60 minutes at room temperature. Remove the plate cover and read the absorbance at a wavelength of 410 nm.
8. If reading kinetically, measure the absorbance every two minutes at room temperature for one hour. Determine the initial rate based on the linear portion of the kinetic curve. Calculations for a kinetic reading can be performed as shown on page 14, substituting initial rates for average absorbance.

ANALYSIS

Calculations

1. Subtract the average absorbance of the background wells from the average absorbance of the vehicle, test compound, and control inhibitor wells. These are the corrected values.
2. Using the corrected values, determine the percent inhibition or percent activity for each test compound using one of the following equations:

$$\% \text{ inhibition} = \left[1 - \frac{\text{corrected value of test compound}}{\text{corrected value of vehicle}}\right] \times 100$$

$$\% \text{ activity} = \frac{\text{corrected value of test compound}}{\text{corrected value of vehicle}} \times 100$$

3. Graph the percent inhibition or percent activity as a function of test compound concentration to determine the IC_{50} value (the concentration at which there is 50% inhibition) of the inhibitor. Inhibition of $sPLA_2$ type IIA by $sPLA_2$ Control Inhibitor is shown in Figure 2 (see page 16).

Performance Characteristics

Z´ Factor

Z´ factor is a term used to describe the robustness of an assay, which is calculated using the equation below.[7]

$$Z' = 1 - \frac{3\sigma_{c+} + 3\sigma_{c-}}{|\mu_{c+} - \mu_{c-}|}$$

Where σ: Standard deviation
μ: Mean
c+: Positive control
c-: Negative control

The theoretical upper limit for the Z' factor is 1.0. A robust assay has a Z' factor >0.5. The Z' factor for Cayman's $sPLA_2$ (Type IIA) Inhibitor Screening Assay Kit was determined to be 0.83.

Sample Data:

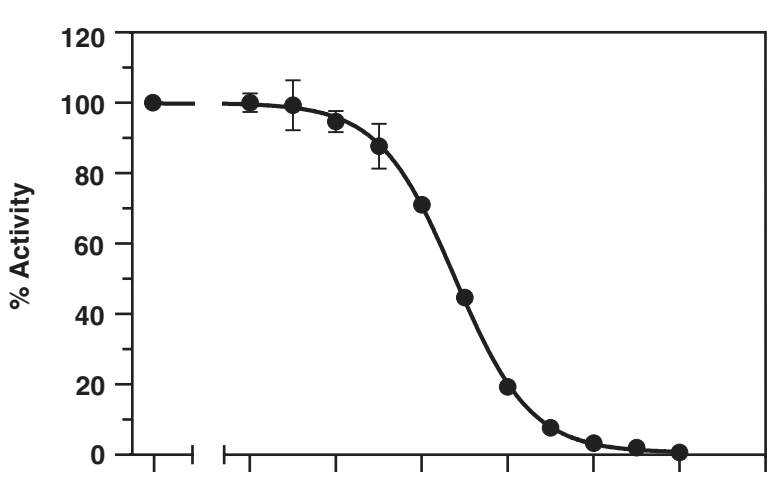

Figure 2. Inhibition of $sPLA_2$ type IIA by $sPLA_2$ Control Inhibitor. Data are plotted as the mean of triplicate measurements ± the standard deviation. The vehicle control (Veh.) represents 100% activity. The IC_{50} value of $sPLA_2$ Control Inhibitor in this experiment is 24 nM.

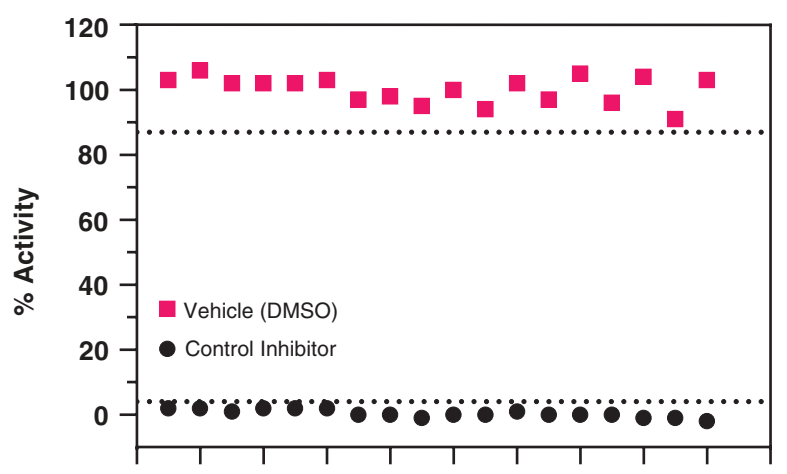

Figure 3. Typical performance data for the $sPLA_2$ (Type IIA) Inhibitor Screening Assay Kit. Data shown are from eighteen replicates each for vehicle and the 28.6 µM $sPLA_2$ Control Inhibitor prepared as described in the kit booklet. The calculated Z′ factor for this experiment was 0.83. The dotted lines correspond to three standard deviations from the mean for each control value.

Effects of Solvents:

Compounds may be prepared in organic solvents such as DMSO, DMF, or short-chain alcohols (*e.g.*, MeOH, EtOH). A titration of organic solvents showed that signal decreases with increasing solvent concentration, so the proper vehicle control should be included in the assay.

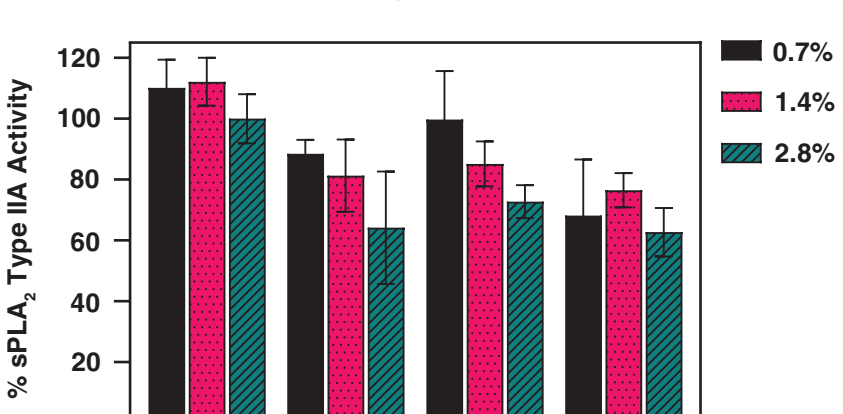

Figure 4. The effect of solvent on the readout of $sPLA_2$ type IIA activity. The data are shown as the mean ± standard deviation for quadruplicate reactions containing the indicated concentration of solvents.

Precision:

Intra-assay precision was determined by analyzing 24 measurements of the background and vehicle and 20 measurements of the control inhibitor on the same day. The intra-assay coefficients of variation were 4, 7, and 3%, respectively. The intra-assay coefficient of variation for the IC_{50} value of 17 inhibition curves performed on the same day was 13%.

Inter-assay precision was determined by analyzing inhibition with the control inhibitor in separate assays on six different days. The inter-assay coefficient of variation for the IC_{50} value was 10%.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of triplicates/ quadruplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
No absorbance above background is seen in the wells	A. Enzyme, probe, or substrate was not added to the well(s) or the enzyme has degraded. B. All of the enzyme activity is inhibited	A. Make sure to add all components to the wells. Use the enzyme master mix immediately or store it on ice for not more than two hours. B. Reduce the concentration of the inhibitor and test again
No inhibition seen with test compounds	A. The concentration of the test compound is not high enough B. The compound is not an inhibitor of the enzyme	Increase the concentration of the test compound and test again

Procedure	Background Wells	Vehicle Wells	Test Wells	Positive Control Wells
Add Background Master Mix	58 µl	--	--	--
Add $sPLA_2$ (Type IIA) Master Mix	--	58 µl	58 µl	58 µl
Add solvent	2 µl	2 µl	--	--
Add 1 mM $sPLA_2$ Control Inhibitor	--	--	--	2 µl
Add test compound	--	--	2 µl	--
Incubate	Cover with the 96-Well Cover Sheet and incubate for 15 minutes at room temperature.			
Add resuspended $sPLA_2$ (Type IIA) Substrate	10 µl			
Mix, Incubate, and Read	Mix the contents well by pipetting up and down 4-5x without introducing bubbles. Measure the absorbance at least every two minutes at room temperature for 60 minutes for a kinetic measurement OR cover with the 96-Well Cover Sheet and incubate for 60 minutes at room temperature for an endpoint measurement. Read absorbance at 410 nm.			

Table 2. Assay summary

References

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2. Kuefner, M.S., Stephenson, E., Savikj, M., *et al.* Group IIA secreted phospholipase A2 (PLA2G2A) augments adipose tissue thermogenesis. *FASEB J.* **35(10)**, e21881 (2021).
3. Cupillard, L., Koumanov, K., Mattéi, M.G., *et al.* Cloning, chromosomal mapping, and expression of a novel human secretory phospholipase A_2. *J. Biol. Chem.* **272(25),** 15745-15752 (1997).
4. Scott, K.F., Mann, T.J., Fatima, S., *et al.* Human group IIA phospholipase A2-Three decades on from its discovery. *Molecules* **26(23)**, 7267 (2021).
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6. Ge, W., Yue, M., Lin, R., *et al.* PLA2G2A$^+$ cancer-associated fibroblasts mediate pancreatic cancer immune escape via impeding antitumor immune response of CD8$^+$ cytotoxic T cells. *Cancer Lett.* **558**, 216095 (2023).
7. Zhang J-H., Chung, T.D.Y., and Oldenburg, K.R. A Simple parameter for use in evaluation and validation of high throughput screening assays. *J. Biomol. Screen.* **4(2)**, 67-73 (1999).

NOTES

Warranty and Limitation of Remedy

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