$sPLA_2$ (human Type IIA) ELISA Kit

Item No. 501380

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size
401382	Anti-sPLA$_2$ (human Type IIA) ELISA Strip Plate	1 plate
401381	Anti-sPLA$_2$ (human Type IIA) HRP Conjugate	1 vial/1.5 ml
401384	sPLA$_2$ (recombinant human Type IIA) ELISA Standard	2 vials/0.5 ml
400060	ELISA Buffer Concentrate (10X)	2 vials/10 ml
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml
400035	Polysorbate 20	1 vial/3 ml
400012	96-Well Cover Sheet	1 cover
400074	TMB Substrate Solution	1 vial/12 ml
10011355	HRP Stop Solution	1 vial/12 ml

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.

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's $sPLA_2$ (human Type IIA) ELISA Kit. 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 at -20°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 450 nm.
2. Adjustable pipettes and a repeating pipettor.
3. A source of pure water; glass distilled water or deionized water is acceptable.
4. Materials used for **Sample Preparation** (see page 10).

INTRODUCTION

Background

Phospholipases A_2 (PLA_2s) make up a superfamily of enzymes that hydrolyze the ester bond of phosphoglycerides at the *sn*-2 position to release free fatty acid and lysophospholipids. This superfamily is divided into three groups by molecular weight and Ca^{2+}-dependence. Secreted PLA_2s ($sPLA_2$s) are small ~14 kDa enzymes that require millimolar concentrations of Ca^{2+}. This family of enzymes is further subdivided based on the number of intramolecular disulfide bonds, and the presence or absence of a C-terminal extension and a three amino acid elapid loop (residues 54-56).[1]

$sPLA_2$s are found in the venom of certain snakes (Types IA, IIA, and IIB from vipers, cobras, rattlesnakes, and kraits), in pancreatic juices (Type IB), in rat and murine testes (Type IIC), in placenta, synovial fluids and platelets (Type IIA), and in heart, placenta, lung, mast cells, and P388D1 macrophages (Type V).[1] Until 1994, the $sPLA_2$ responsible for the release of arachidonic acid in inflammation was believed to be Type IIA. Although recently discovered isoforms of PLA_2 clearly contribute to the release of arachidonic acid, $sPLA_2$ (Type IIA) continues to be a protein of interest in the field of inflammation.

This ELISA is specific for Type IIA $sPLA_2$, and does not cross react with Type I, Type IV, Type V PLA_2, inflammatory mediators such as tumor necrosis factor, interleukin-1, or platelet-activating factor.

About This Assay

Cayman's $sPLA_2$ (human Type IIA) ELISA Kit is a sandwich assay that can be used for quantification of $sPLA_2$ in plasma, synovial fluid, and other sample matrices. The ELISA has a range from 31.3-2,000 pg/ml, with a limit of quantification of 31.3 pg/ml.

Description of Sandwich ELISAs

This immunometric assay is based on a double-antibody 'sandwich' technique. Each well of the microwell plate supplied with the kit has been coated with a monoclonal antibody specific for $sPLA_2$ (human Type IIA) Capture Antibody). This antibody will bind any $sPLA_2$ (human Type IIA) introduced into the well. Standards or biological test samples are incubated on the antibody-coated plate, and the plate is then rinsed before addition of a second, non-overlapping HRP-conjugated mouse monoclonal antibody specific for $sPLA_2$ (human Type IIA) that is used to detect the captured $sPLA_2$ (human Type IIA). The concentration of the $sPLA_2$ (human Type IIA) is determined by measuring the enzymatic activity of HRP using the chromogenic substrate 3,3',5,5'-tetramethylbenzidine (TMB). After a sufficient period of time, the reaction is stopped with acid, forming a product with a distinct yellow color that can be measured at 450 nm. The intensity of this color, determined spectrophotometrically, is directly proportional to the amount of bound HRP-streptavidin conjugate, which in turn is proportional to the concentration of the $sPLA_2$ (human Type IIA).

$$\text{Absorbance} \propto [\text{HRP-sPLA}_2\ \text{mAb}] \propto [\text{Total sPLA}_2\ \text{(human Type IIA)}]$$

A schematic description of the assay is given in Figure 1 on page 8.

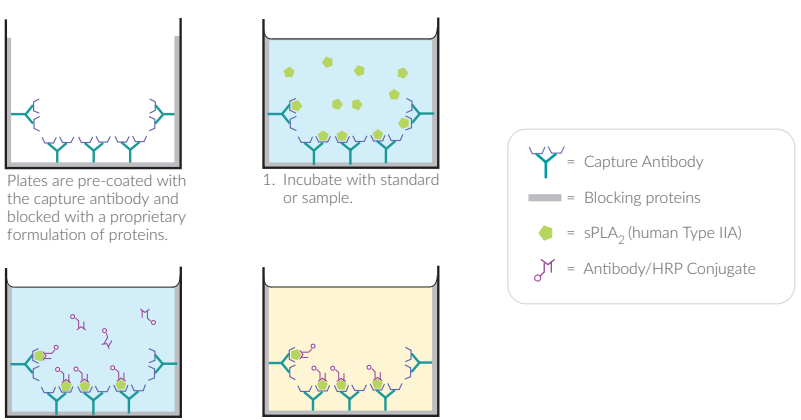


Figure 1. Schematic of the ELISA

Definition of Key Term

Standard Curve: a plot of the absorbance values *versus* concentration of a series of wells containing various known amounts of free analyte.

PRE-ASSAY PREPARATION

NOTE: Water used to prepare all ELISA reagents and buffers must be deionized and free of trace organic contaminants ('UltraPure'). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for ELISA. UltraPure water may be purchased from Cayman (Item No. 400000).

Buffer Preparation

Store all diluted buffers at 4°C; they should be stable for two months.

1. **ELISA Buffer Preparation**

   Dilute the contents of one vial of ELISA Buffer Concentrate (10X) (Item No. 400060) with 90 ml of UltraPure water. Be certain to rinse the vial to remove any salts that may have precipitated. *NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with water.*

2. **Wash Buffer Preparation**

   5 ml vial Wash Buffer Concentrate (400X): Dilute to a total volume of 2 liters with UltraPure water and add 1 ml of Polysorbate 20 (Item No. 400035). *NOTE: Polysorbate 20 is a viscous liquid and cannot be measured by a regular pipette. A positive displacement pipette or a syringe should be used to deliver small quantities accurately.*

Sample Preparation

In general, samples can be assayed with no prior purification. We recommend diluting plasma at least 1:20 and synovial fluid at least 1:1,000 into ELISA Buffer.

General Precautions

- All samples must be free of organic solvents prior to assay.
- Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C.

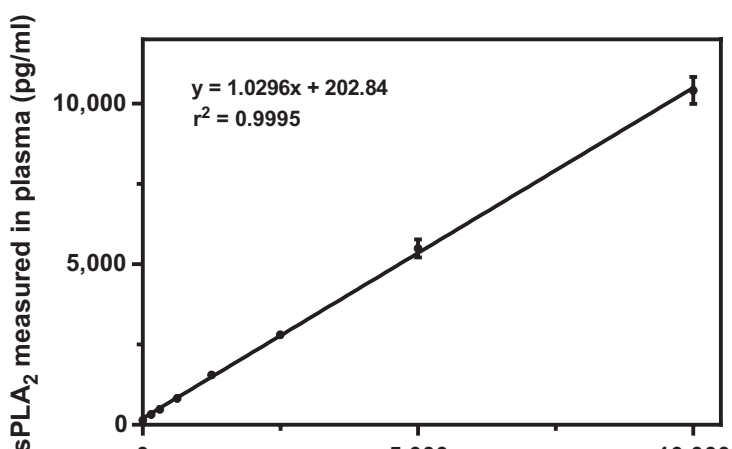


Figure 2. $sPLA_2$ (human Type IIA) recovery in plasma
Plasma samples were spiked with $sPLA_2$ (human Type IIA), diluted as described in the figure, and analyzed by ELISA.

Preparation of Assay-Specific Reagents

$sPLA_2$ (human Type IIA) ELISA Standard

Thaw the $sPLA_2$ (human Type IIA) ELISA Standard (Item No. 401384) at room temperature. The concentration of this solution is 10 ng/ml. Store this solution at 4°C; it should be stable for approximately four weeks. Enough $sPLA_2$ is included to run twenty standard curves. This surplus should accommodate any experimental design.

To prepare the standard for use in ELISA: Obtain eight clean test tubes and label them #1 through #8. Aliquot 800 µl ELISA Buffer to tube #1 and 500 µl ELISA Buffer to tubes #2-8. Transfer 200 µl of the bulk standard (10 ng/ml) to tube #1 and mix thoroughly. The $sPLA_2$ concentration of this tube is 2,000 pg/ml and is the highest point on the standard curve. Serially dilute the standard by removing 500 µl from tube #1 and placing in tube #2; mix thoroughly. Next, remove 500 µl from tube #2 and place into tube #3; mix thoroughly. Repeat this process for tubes #4-7. Do not add any $sPLA_2$ to tube #8. This tube is the zero-point vial, the lowest point on the standard curve. These diluted standards should not be stored for more than 24 hours.

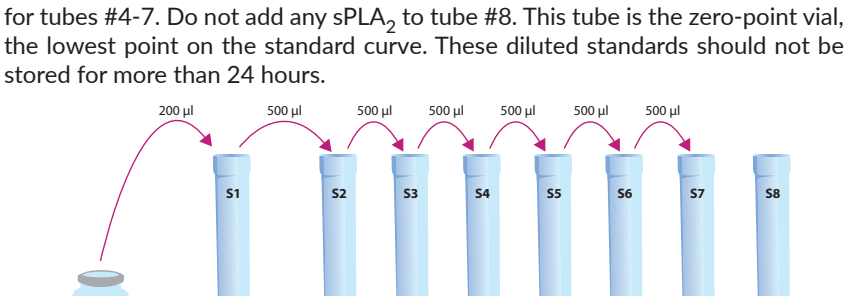


Figure 3. Preparation of the $sPLA_2$ (human Type IIA) standards

Anti-$sPLA_2$ (human Type IIA) HRP Conjugate

This reagent is supplied as a concentrated (10X) stock solution of $sPLA_2$ (human Type IIA) antibody conjugated to HRP. On the day of the assay, thaw the reagent (Item No. 401381) at room temperature. For a full plate, dilute 1.2 ml of HRP Conjugate into 10.8 ml of 1X ELISA Buffer (Item No. 400060); for a half plate, dilute 0.6 ml of HRP Conjugate into 5.4 ml of 1X ELISA Buffer. Do not prepare diluted HRP Conjugate until immediately before use. The concentrated (10X) stock solution can be stored at 4°C after thawing; it should be stable for approximately four weeks.

Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. *NOTE: If you do not need to use all the strips at once, place the unused strips back in the plate packet and store at 4°C. Be sure the packet is sealed with the desiccant inside.*

Each plate or set of strips must contain an eight point standard curve run in duplicate. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results.* Each sample should be assayed at a minimum of two dilutions and each dilution should be assayed in duplicate. For statistical purposes, we recommend assaying samples in triplicate.

A suggested plate format is shown in Figure 4, below. The user may vary the location and type of wells present as necessary for each particular experiment. The plate format provided below has been designed to allow for easy data analysis using a convenient spreadsheet offered by Cayman (see **Analysis**, page 17, for more details). We suggest you record the contents of each well on the template sheet provided on page 22.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	1	1	1	9	9	9	17	17	17	25
B	S2	S2	2	2	2	10	10	10	18	18	18	25
C	S3	S3	3	3	3	11	11	11	19	19	19	25
D	S4	S4	4	4	4	12	12	12	20	20	20	26
E	S5	S5	5	5	5	13	13	13	21	21	21	26
F	S6	S6	6	6	6	14	14	14	22	22	22	26
G	S7	S7	7	7	7	15	15	15	23	23	23	
H	S8	S8	8	8	8	16	16	16	24	24	24	

S1-S8 - Standards 1-8
1-26 - Samples

Figure 4. Sample plate format

Performing the Assay

Pipetting Hints

- Use different tips to pipette the buffer, standard, sample, and conjugate.
- 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.

Addition of sPLA2 (human Type IIA) ELISA Standard and First Incubation

1. Add 100 µl of the standards or diluted sample to the appropriate wells on the plate. Each sample should be assayed in duplicate, triplicate recommended.
2. Cover the plate with a 96-Well Cover Sheet (Item No. 400012). Incubate for two hours at room temperature on an orbital shaker.

Addition of Anti-sPLA$_2$ (human Type IIA) HRP Conjugate and Second Incubation

1. Empty the wells and rinse four times with Wash Buffer. Each well should be completely filled with Wash Buffer during each wash. Invert the plate between wash steps to empty the fluid from the wells. After the last wash, gently tap the inverted plate on absorbent paper to remove the residual Wash Buffer.
2. Add 100 µl of the Anti-sPLA$_2$ (human Type IIA) HRP working solution to each well of the plate.
3. Cover the plate with a 96-Well Cover Sheet and incubate for one hour at room temperature on an orbital shaker.

Development the Plate

1. Empty the wells and rinse four times with Wash Buffer as described above.
2. Add 100 µl of TMB Substrate Solution (Item No. 400072) to each well of the plate.
3. Cover the plate with a 96-Well Cover Sheet and incubate for 30 minutes at room temperature in the dark. Development of the blue color can be monitored at 650 nm.
4. *DO NOT WASH THE PLATE*. Add 100 µl of HRP Stop Solution (Item No. 10011355) to each well of the plate. Blue wells should turn yellow and colorless wells should remain colorless. *NOTE: The Stop Solution in this kit contains an acid. Wear appropriate protection and use caution when handling this solution.*

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc.
2. Read the plate at a wavelength of 450 nm.

ANALYSIS

Many plate readers come with data reduction software that plots data automatically. Alternatively a spreadsheet program can be used. *NOTE: Cayman Chemical has a computer spreadsheet available for data analysis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/immuno) to obtain a free copy of this convenient data analysis tool.*

Calculations

Plotting the Standard Curve and Determining the Sample Concentration

Using computer reduction software, plot absorbance (linear y-axis) *versus* concentration (linear x-axis) for standards (S1-S8) and fit the data with a 4-parameter logistic equation. Using the equation of the line, calculate the concentration of $sPLA_2$ in each sample.

Performance Characteristics

Sensitivity:

The Lower Limit of Quantification (LLOQ) of the assay is 31.3 pg/ml.

The Lower Limit of Detection (LLOD) of the assay is 9.3 pg/ml.

Sample Data

The standard curve presented (see page 19) is an example of the data typically obtained with this kit; however, your results will not be identical to these. You must run a new standard curve. Do not use the data below to determine the values of your samples. Your results could differ substantially.

$sPLA_2$ (pg/ml)	Raw O.D. 450 nm	%CV Intra-Assay Precision	%CV Inter-Assay Precision
2,000	1.958	2.2	8.9
1,000	1.019	1.9	8.9
500	0.564	1.7	8.7
250	0.336	2.8	9.7
125	0.231	1.5	7.1
62.5	0.169	2.7	3.4
31.3	0.143	3.3	5.2
0	0.116	5.8	8.7

Table 1. Representative data

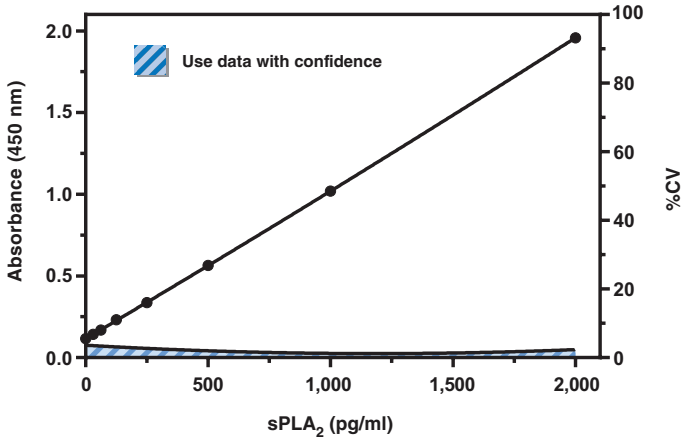


Figure 5. Typical standard curve

Precision:

Intra-assay precision was determined by analyzing at least 20 replicates of 3 matrix controls (human plasma samples) in a single assay.

Matrix Control (pg/ml)	%CV*
1,641.7	2.7
827.0	4.0
248.3	4.5

Table 2. Intra-assay Precision

Inter-assay precision was determined by analyzing replicates of 3 matrix controls (human plasma samples) in separate assays spanning across several days.

Matrix Control (pg/ml)	%CV*
1,459.1	10.5
710.4	15.0
289.9	14.6

Table 3. Inter-assay Precision

Cross Reactivity:

This kit does not recognize PLA_2 Type I, IV, V, and VI.

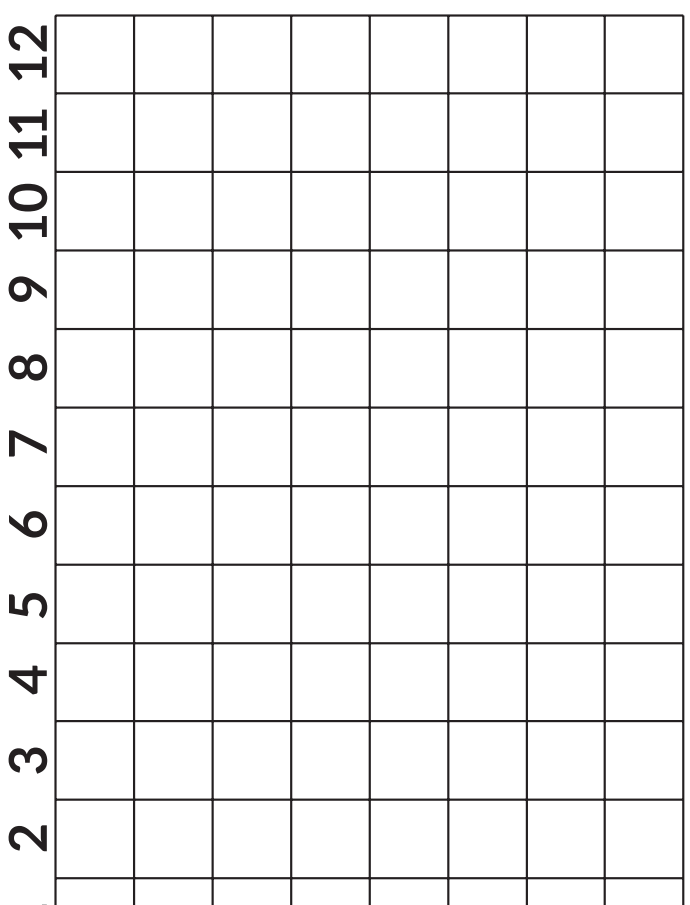
RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Poor development (low signal) of standard curve	A. Standard was diluted incorrectly B. 10X HRP Conjugate was diluted into ELISA Buffer for too long before use, resulting in inactivation of the HRP C. Standard is degraded	Perform assay again with a new aliquot of Standard or HRP Conjugate
Analyses of two dilutions of a biological sample do not agree (*i.e.*, more than 20% difference)	Interfering substances are present	Sample must be purified prior to analysis by ELISA[2]
Sample concentrations appear inconsistent with literature values	Matrix for samples and standards are different	Use same matrix for all samples and standards

References

1. Dennis, E.A. The growing phospholipase A_2 superfamily of signal transduction enzymes. *Trends Biochem. Sci.* **22**, 1-2 (1997).
2. Maxey, K.M., Maddipati, K.R. and Birkmeier, J. Interference in enzyme immunoassays. *J. Clin. Immunoassay* **15**, 116-120 (1992).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.