

Version 4a Last updated 13 June 2025

# ab273278 Phospholipase A2 Activity Assay Kit (Fluorometric)

View Kit datasheet: <https://www.abcam.com/ab273278>  
(use <https://www.abcam.cn/ab273278> for china, or  
<https://www.abcam.co.jp/ab273278> for Japan)

For the measurement of phospholipase A2 activity in various tissues/cells, venoms, secretory fluids.

This product is for research use only and is not intended for diagnostic use.

## Table of Contents

1. Overview	1
2. Protocol Summary	2
3. Precautions	3
4. Storage and Stability	3
5. Limitations	4
6. Materials Supplied	4
7. Materials Required, Not Supplied	5
8. Technical Hints	6
9. Reagent Preparation	7
10. Sample Preparation	8
11. Standard Curve	9
12. Assay Procedure	10
13. Calculations	12
14. Typical Data	13
15. FAQ / Troubleshooting	15
16. Notes	16

## 1. Overview

Phospholipase A2 Activity Assay Kit (Fluorometric) (ab273278) provides a quick, sensitive, and simple way for measuring PLA2 activity in various biological samples. In this assay, active PLA2 cleaves a synthetic thiophospholipid, producing a lysothiophospholipid which reacts with a fluorogenic probe to produce a highly fluorescent product detectable in the visible range (Ex/Em= 388/513 nm). The assay is simple to perform, high-throughput adaptable and can detect less than 0.1 mU of PLA2 activity.

## 2. Protocol Summary

Prepare samples as directed



Prepare all reagents as directed



Prepare standard curve and measure fluorescence at Ex/Em = 388/513 nm.



Add Positive Control, Samples, Background Control to appropriate wells and adjust volume to 50  $\mu$ L



Add PLA2 Probe (10  $\mu$ L)



Add PLA2 Substrate (40  $\mu$ L) to Positive Control, Samples and PLA2 Assay buffer (40  $\mu$ L) to Background Control



Measure fluorescence (Ex/Em = 388/513 nm) in kinetic mode for 45 to 60 minutes at 37°C.

### 3. Precautions

**Please read these instructions carefully prior to beginning the assay.**

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink, or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

### 4. Storage and Stability

**Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 6 months from receipt.**

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

## 5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

## 6. Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
PLA2 Assay Buffer	25 mL	-20°C	-20°C
PLA2 Substrate	40 µL	-20°C	-20°C
PLA2 Probe	100 µL	-20°C	-20°C
PLA2 Fluorescence Standard	1 vial	-20°C	-20°C
Bee Venom Positive Control	1 vial	-20°C	-20°C

PLEASE NOTE: PLA2 Fluorescence Standard was previously labelled as Fluorescence Standard (Lyophilized). The composition has not changed.

## 7. Materials Required, Not Supplied

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

- Multi-well spectrophotometer capable of measuring fluorescence at Ex/Em= 388/513 nm
- Black 96-well plate with flat bottom
- 30 kDa Spin Columns
- DMSO

## 8. Technical Hints

- **This kit is sold based on number of tests. A “test” simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

## 9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

### 9.1 PLA2 Assay Buffer:

Store at -20 °C. Bring to room temperature before use.

### 9.2 PLA2 Substrate:

Thaw before use. Aliquot as desired and store at -20°C. While using, keep on ice after dilution. Avoid repeated freeze/thaw cycles. Use within two months.

### 9.3 PLA2 Probe:

Thaw before use. Aliquot and store at -20°C.

### 9.4 PLA2 Fluorescence Standard:

Reconstitute with 110 µL DMSO to generate 1 mM Standard solution. Aliquot and store at -20°C. Allow to thaw to room temperature before use and keep at room temperature while in use. Use within two months.

### 9.5 Bee Venom Positive Control:

Reconstitute with 22 µL PLA2 Assay Buffer and mix thoroughly. Aliquot and store at -20°C. Use within two months. Keep on ice while in use.

## 10. Sample Preparation

### 10.1 Tissue/cell lysate preparation:

- 10.1.1 Rapidly homogenize tissue (10 mg) or cells ( $1 \times 10^6$ ) with 100  $\mu$ L PLA2 Assay Buffer and keep on ice for 10 minutes.
- 10.1.2 Centrifuge at 10,000  $\times g$  for 10-15 minutes at 4°C and transfer the supernatant to a fresh, prechilled microfuge tube.

**Δ Note:** Complex biological samples such as tissue homogenates and cell lysates may contain a mixture of PLA2 subtypes; detected sample activity, therefore reflects total PLA2 activity. The activity of low molecular weight (secretory) Phospholipase A2 enzymes (sPLA2s) may be determined by ultrafiltration of the sample using 30 kDa MWCO spin columns to remove any high molecular weight (cytosolic) phospholipase A2 enzymes (cPLA2s and iPLA2s). Add sample to the spin column, centrifuge at 10,000  $\times g$  at 4°C for 10 minutes and collect the filtrate, which will contain only sPLA2s.

**Δ Note:** For unknown samples, we suggest doing a pilot experiment to ensure readings are within the range of the standard curve. Samples with extremely high PLA2 activity (such as venoms and other secretory fluids) may be diluted with PLA2 Assay Buffer.

**Δ Note:** We recommend using a protease inhibitor cocktail to prevent enzyme degradation and measuring sample protein concentration using Bradford reagent or a comparable protein assay.

**Δ Note:** For samples exhibiting significant background, prepare parallel sample well(s) as background controls.

## 11. Standard Curve

- 11.1 Prepare a 0.1 mM solution of PLA2 Fluorescence Standard by diluting the PLA2 Fluorescence Standard stock solution 10 times with dH<sub>2</sub>O just prior to use.
- 11.2 Add 0, 2, 4, 6, 8 and 10  $\mu$ L of the 0.1 mM PLA2 Fluorescence Standard into a series of wells in a 96 well plate and adjust the volume to 100  $\mu$ L/well with PLA2 Assay Buffer. This will generate 0, 0.2, 0.4, 0.6, 0.8 and 1 nmol/well of PLA2 Fluorescence Standard respectively.

Standard #	PLA2 Fluorescence Standard ( $\mu$ L)	PLA2 Assay Buffer ( $\mu$ L)	PLA2 Fluorescence Standard (nmol/well)
1	10	90	1.0
2	8	92	0.8
3	6	94	0.6
4	4	96	0.4
5	2	98	0.2
6	0	100	0

- 11.3 Mix well and measure the fluorescence (Ex/Em = 388/513 nm) in an end point mode.

## 12. Assay Procedure

Thaw all reagents thoroughly and mix gently.

- 12.1.1 For Sample (S), add 5-25  $\mu\text{L}$  sample per well in a black 96 well plate and adjust the volume to 50  $\mu\text{L}$  with PLA2 Assay Buffer.
- 12.1.2 For Positive Control, dilute the required amount of Bee Venom Positive Control 100 times with PLA2 Assay Buffer. Add 10  $\mu\text{L}$  of the diluted Bee Venom Positive Control per well into the desired well(s) and adjust the final volume to 50  $\mu\text{L}$  with PLA2 Assay Buffer. Use the diluted positive control solution within one hour of preparation.
- 12.1.3 Dilute the PLA2 Probe stock solution 10 times with  $\text{dH}_2\text{O}$  to obtain a 1X working solution. Add 10  $\mu\text{L}$  of the 1X working solution of PLA2 Probe to all the wells except those containing the standards.
- 12.1.4 Dilute the PLA2 Substrate stock solution 100 times with PLA2 Assay Buffer to obtain a working solution. Make enough to add 40  $\mu\text{L}$  per well (for example, for 10 reactions, add 4  $\mu\text{L}$  of PLA2 Substrate stock to 396  $\mu\text{L}$  PLA2 Assay Buffer to make 400  $\mu\text{L}$  of working solution). Vortex.
- 12.1.5 Add 40  $\mu\text{L}$  PLA2 Substrate working solution to each test and positive control well.

**Δ Note:** Do not add PLA2 Substrate to the Standard wells or to the Background Control wells.

Component	Test Sample/Positive Control	Sample Background Control
Substrate Solution (1X)	40 $\mu\text{L}$	--
PLA2 Assay Buffer	--	40 $\mu\text{L}$

**Δ Note:** The PLA2 probe solution (1X) and the Substrate solution (1X) should be added to the wells sequentially as per protocol and not premixed as a single solution.

- 12.1.6 Measure fluorescence ( $\text{Ex/Em} = 388/513 \text{ nm}$ ) in kinetic mode for 45-60 minutes at 37°C.

**Δ Note:** Measurement time for the linear phase of the reaction depends on the PLA2 activity in samples. We recommend measuring the sample fluorescence in kinetic mode to ensure that the measurements recorded are within the linear range of the reaction.

## 13. Calculations

- 13.1 Subtract the 0 nmol PLA2 Fluorescence Standard reading from all standard curve readings, plot the standard curve and calculate the slope.
- 13.2 For each reaction well (including sample background and positive control wells), choose two time points ( $t_1$  and  $t_2$ ) in the linear phase, obtain the corresponding fluorescence values at those points ( $RFU_1$  and  $RFU_2$ ) and determine the change in fluorescence over the time interval:  $\Delta F = RFU_2 - RFU_1$ .
- 13.3 If sample background control reading is significant, subtract the background control reading from its paired sample reading.
- 13.4 PLA2 metabolic activity is obtained by applying the background-corrected  $\Delta F$  values to the PLA2 Fluorescence Standard curve to get B nmol of PLA2 Substrate metabolized during the reaction time ( $\Delta T = T_2 - T_1$ ).

$$\text{Sample Phospholipase A2 Activity} = \frac{B}{(\Delta T \times V)} \times D = \text{nmol/min/mL} = \text{mU/mL}$$

B = metabolite amount from standard curve (nmol).

$\Delta t$  = Reaction time of linear phase ( $t_2 - t_1$ ) (min)

V = sample volume added into the reaction well (ml).

D = Sample dilution factor (if applicable)

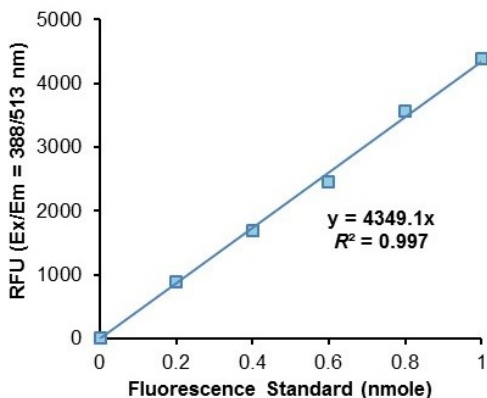
### Unit definition:

One unit of Phospholipase A2 is the amount of enzyme that generates 1.0  $\mu\text{mole}$  of lysothiophospholipid metabolite per minute at pH 7.5 at 37°C.

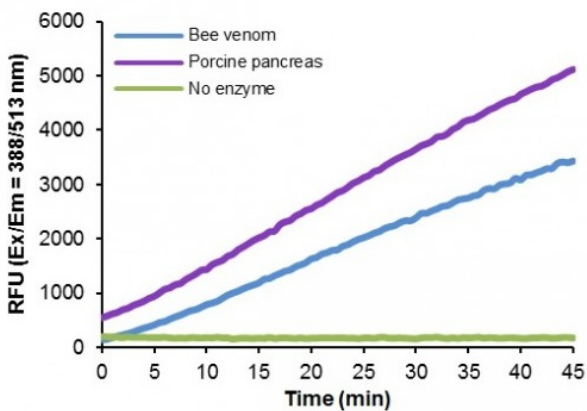
**$\Delta$  Note:** Activity of the high molecular weight Phospholipase A2 enzymes (cPLA2 and iPLA2 type enzymes) can be calculated by subtracting the activity of the secretory Phospholipase A2 enzymes (filtered fraction) from the total Phospholipase A2 activity.

## 14. Typical Data

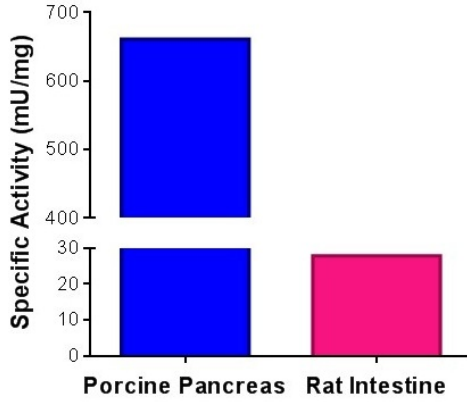
Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.



**Figure 1.** PLA2 Fluorescence Standard curve. One mole of PLA2 Fluorescence Standard corresponds to the metabolism of one mole of PLA2 Substrate.



**Figure 2.** Reaction kinetics of Phospholipase A2 activity in bee venom positive control and porcine pancreas (0.314  $\mu\text{g}$  protein) at 37°C using appropriate background controls.



**Figure 3.** Phospholipase A2 specific activity calculated in porcine pancreas and rat intestine. Assays were performed following the kit protocol.

## 15. FAQ / Troubleshooting

General troubleshooting points are found at [www.abcam.com/assaykitguidelines](http://www.abcam.com/assaykitguidelines).

## 16. Notes



## Technical Support

Copyright © 2025 Abcam. All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.

For all technical or commercial enquiries please go to:

[www.abcam.com/contactus](http://www.abcam.com/contactus)

[www.abcam.cn/contactus](http://www.abcam.cn/contactus) (China)

[www.abcam.co.jp/contactus](http://www.abcam.co.jp/contactus) (Japan)