

EnzChek® Phospholipase A₂ Assay Kit

Catalog nos. E10217, E10218

Table 1. Contents and storage information.

Material	E10217 (2-plates)	E10218 (10-plates)	Concentration	Storage	Stability
Phospholipase A ₂ substrate (Red/Green BODIPY® PC-A2) (Component A)	2 vials	10 vials	Not applicable	<ul style="list-style-type: none"> • ≤-20°C • Desiccate • Protect from light 	When stored as directed the product is stable for at least 1 year.
Dioleoylphosphatidylcholine (DOPC) (Component B); MW = 785.59	800 µg	4 mg		<ul style="list-style-type: none"> • ≤-20°C • Desiccate 	
Dioleoylphosphatidylglycerol (DOPG) (Component C); MW = 797.04	800 µg	4 mg			
Phospholipase A ₂ from honey bee venom (Component D)	40 Units	200 Units			
Dimethylsulfoxide (DMSO, Component E)	200 µL	1 mL		Desiccate	
5X Phospholipase A ₂ reaction buffer (Component F)	10 mL	50 mL	250 mM Tris-HCl, 500 mM NaCl, 5 mM CaCl ₂ , pH 8.9	≤-20°C	

Number of assays: For Cat. no. E10217, sufficient material is supplied for 200 reactions in 96-well microplates at a volume of 100 µL per well as described in the protocol below, or 800 reactions using low-volume 384-well microplates at a volume of ≤25 µL per well. For Cat. no. E10218, sufficient material is supplied for 1,000 reactions in 96-well microplates at a volume of 100 µL per well as described in the protocol below or 4,000 reactions using low-volume 384-well microplates at a volume of ≤25 µL per well.

Approximate fluorescence excitation/emission maxima: Excitation = 505 nm (typical plate reader setting Ex ≈ 460 nm); Emission = 515 nm (intensity based); Emission = 515/575 nm (ratiometric).

Introduction

The EnzChek® Phospholipase A₂ Assay Kit provides a simple fluorometric method designed for continuous monitoring of phospholipase A₂ (PLA₂). PLA₂ represents a family of enzymes that hydrolyze the *sn*-2 ester linkage of phospholipids and play important roles in cardiovascular, inflammatory and nervous system disorders, and cancers. The EnzChek® Phospholipase A₂ substrate is selective for PLA₂, and provides sensitive and continuous rapid real-time monitoring of PLA₂ enzyme activities for rapid and direct analysis of PLA₂ using automated instruments. (Figure 1). The enzyme assay can be used in a intensity or ratiometric based detection mode providing a simple method with low background, high sensitivity, and high specificity for PLA₂. In intensity-based detection mode, the PLA₂ activity is monitored by the intensity increase of a single wavelength at

approximately 515 nm. In ratiometric analysis, which is based on the distinct fluorescence resonance energy transfer (FRET) emission of the substrate prior to and after cleavage, PLA₂ is detected by changes in the emission intensity ratio at 515/575 nm with excitation at ~460 nm.

The EnzChek® Phospholipase A₂ Assay Kit can detect bee venom PLA₂ at 0.05 Units/mL or lower (see Figure 2). The kit may be amenable for use with cells or cell lysates and for characterizing PLA₂ inhibition (Figure 3).

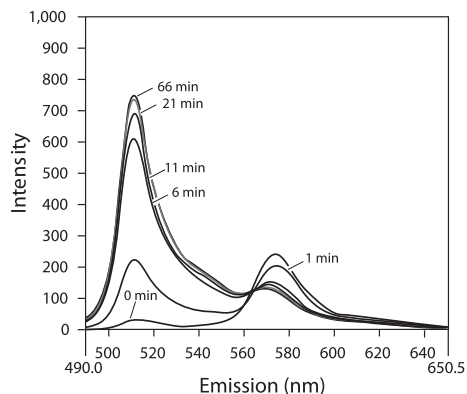


Figure 1. Fluorescence emission spectra of EnzChek® Phospholipase A₂ substrate incorporated in liposomes with addition of bee venom PLA₂ at ambient temperature.

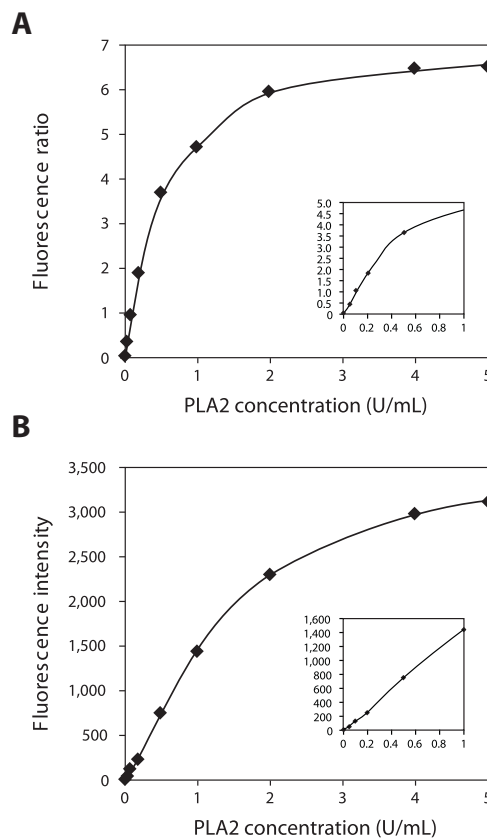


Figure 2. Plot of fluorescence emission intensities versus concentration of PLA₂ per well at 10 minutes, run at ambient temperature with liposomes. Fluorescence was measured exciting at 460 nm on a Spectra Max M5 (Molecular Devices). **Panel A:** Ratiometric (FRET)-based (515/575 nm) EnzChek® Phospholipase A₂ substrate. **Panel B:** Intensity-based detection mode (515 channel)-EnzChek® Phospholipase A₂ substrate. Background fluorescence determined for the no-PLA₂ enzyme control reaction has been subtracted for each value.

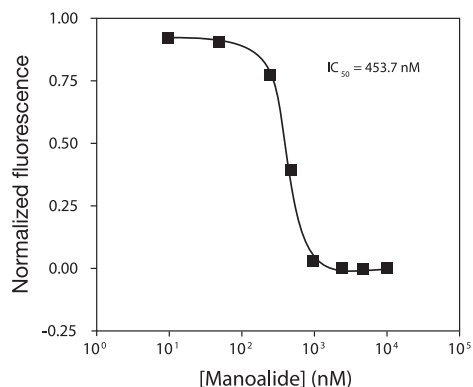


Figure 3. IC₅₀ determination for Manoalide, a known inhibitor¹ using EnzCheck® Phospholipase A₂ Assay Kit. Samples of Manoalide were titrated in the presence of 1.65×10^{-6} M Red/Green BODIPY® PC-A2 and 0.125 U/mL PLA₂ from bee venom in 1X PLA₂ reaction buffer. Reactions were incubated for 5 minutes at room temperature and fluorescence was measured at Ex/Em 460/515 nm using a microplate reader.

Before You Begin

Materials Required but Not Provided

- Samples
- Deionized water
- Ethanol
- Plastic vials for reagent preparation
- Microplates, 96-well or 384-well
- Magnetic stirrer, stir bar, and pipettor (an air displacement pipettor with 100 μ L capacity, fitted with a narrow orifice gel-loading tip is suitable and required for the liposomes preparation at step 2.6)

General Guidelines

- The kit is useful for detecting PLA₂ activity in samples or for screening PLA₂ inhibitors.
- The assay protocol below is optimized for use with 96-well microplates using a 100 μ L reaction volume per assay. For 384-well plates, adjust the reaction volumes accordingly to 25 μ L per assay (recommended).
- The assay protocol is designed for use with a fluorescence microplate reader. A SpectraMax M5 (Molecular Devices) was used throughout the development of this kit.
- Allow the kit components to equilibrate to room temperature before use.
- Use the included PLA₂ reaction buffer for optimal performance.

Preparing Solutions

1.1 1 mM EnzChek® Phospholipase A₂ Substrate: Allow one vial of PLA₂ substrate (Component A) and DMSO (Component E) to warm to room temperature. Dissolve the contents of one vial of PLA₂ substrate in 40 μ L DMSO. One vial of PLA₂ substrate is sufficient for approximately 100 assays with a final reaction volume of 100 μ L per assay.

Store this stock solution frozen at $\leq -20^{\circ}\text{C}$, **protected from light**.

1.2 1X EnzChek® PLA₂ Reaction Buffer: Add 4 mL of 5X PLA₂ reaction buffer (Component F) to 16 mL deionized water. This 20 mL volume of 1X reaction buffer is sufficient for approximately 100 assays of 100 μ L each with 10 mL excess for making stock solutions. Store remaining solution at 4°C.

- 1.3 10 mM DOPC (Dioleoylphosphatidylcholine):** Dissolve the contents of DOPC vial (Component B) in 100 μL (Cat. no. E10217) or 500 μL (Cat. no. E10218) ethanol. Store solution at $\leq -20^{\circ}\text{C}$.
- 1.4 10 mM DOPG (Dioleoylphosphatidylglycerol):** Dissolve the contents of DOPG vial (Component C) in 100 μL (Cat. no. E10217) or 500 μL (Cat. no. E10218) ethanol. Store solution at $\leq -20^{\circ}\text{C}$.
- 1.5 500 Units/mL PLA₂ Stock Solution:** Dissolve contents of the PLA₂ vial (Component D) in 80 μL (Cat. no. E10217, 2 plates) or 400 μL (Cat. no. E10218, 10 plates) of 1X PLA₂ reaction buffer prepared in step 1.2. Sufficient enzyme is supplied to prepare 80 (Cat. no. E10217) or 800 (Cat. no. E10218) positive control samples at 5 U/mL in an assay volume of 100 μL . You may use other sources of PLA₂ as a positive control, but the sensitivity and dynamic range of the assay may be affected. Store stock solution at 4°C.

Experimental Protocol

Perform the standard assay protocol below using a total volume of 100 μL per well. Mix the samples and controls with the substrate-liposome mix at a ratio of 1:1 (50 μL sample/control + 50 μL substrate-liposome mix), such that the concentration of each component is two-fold lower in the final reaction volume. You may also use other volumes may be used, provided that you maintain the ratio of samples/controls to substrate at 1:1.

The protocol was developed and tested using group III PLA₂ from bee venom and Group IB PLA₂ from bovine pancreas.

Assay Protocol

- 2.1** Prepare a PLA₂ standard curve by diluting the appropriate amount of 500 Units/mL PLA₂ stock solution to 10 Units/mL in 1X PLA₂ reaction buffer to produce PLA₂ concentrations of 0–10 Units/mL, each in a volume of 50 μL . Final PLA₂ concentration is two-fold lower (0–5 Units/mL).
- 2.2** If no standard curve is to be used, prepare positive and negative controls. For a positive control, dilute the 500 Units/mL PLA₂ stock solution to 10 Units/mL in 1X PLA₂ reaction buffer. For a negative control, use 1X PLA₂ reaction buffer without PLA₂.
- 2.3** Dilute the PLA₂-containing samples in PLA₂ reaction buffer. You will need 50 μL sample for each reaction. A variable dilution may be required depending on the total PLA₂ present in each sample.
- 2.4** Pipet 50 μL of the standard curve samples (step 2.1) or controls (step 2.2), and experimental samples (step 2.3) into individual wells of a microplate.
- 2.5** Prepare the Lipid Mix by mixing together 30 μL 10 mM DOPC (from step 1.3), 30 μL 10 mM DOPG (from step 1.4), and 30 μL 1 mM PLA₂ substrate (from step 1.1).
- 2.6** Add 5 mL 1X PLA₂ reaction buffer to a 20 mL beaker containing a small magnetic stir bar and place the beaker on a magnetic stirrer to form a vortex. To prepare 5 mL substrate-liposome for 100 assays, slowly and steadily (over about 1 minute) inject 50 μL of Lipid Mix (from step 2.5) into the side of the vortex using a pipettor fitted with a narrow orifice gel-loading tip.
- 2.7** Add 50 μL of the substrate-liposome mix (from step 2.6) to each microplate well containing standards, controls, and samples to start the reaction.

2.8 Incubate at room temperature for 10 minutes, **protected from light**. Because the assay is continuous (not terminated), you may measure fluorescence at multiple time points to follow the kinetics of the reactions.

2.9 Measure the fluorescence using a microplate reader equipped for excitation in the range of 450–490 nm and fluorescence emission at ~515 nm and ~575 nm.

For ratiometric (FRET) detection, use a ratio of the emission 515 nm/575 nm. For single wavelength intensity detection, use emission at ~515 nm (see Figure 2).

2.10 For each point, subtract the value derived from the no-PLA₂ control to correct for background fluorescence.

Inhibitor Screening

For screening PLA₂ inhibitors, select the concentration of enzyme which yields a signal increase at least 20% of maximal.

For IC₅₀ determinations, prepare serial dilutions of the inhibitor(s) of interest and include the selected amount of PLA₂ in the substrate-liposome mix at 2X the final concentration.

Troubleshooting

Problem	Cause	Solution
No response from the control enzyme	Low substrate concentration or substrate is contaminated	Substrate stock solution in DMSO appears purple. If no color is visible, the substrate is too dilute. If color is visible, but there is no response, the substrate is contaminated. Repeat the experiment with fresh substrate.
No response from samples	PLA ₂ absent, inactivated, or is present in low quantities	Increase incubation time or enzyme amount. If no signal, repeat the experiment with a fresh vial of substrate.
Response not in the linear range	PLA ₂ in the sample is highly active	Dilute sample until the response falls within linear range of the standard curve.
DOPG is not in solution	DOPG precipitates from ethanolic solution when stored at -20°C	Redissolve DOPG by warming the solution to room temperature.

Reference

1. Biochem Pharmacol. 36, 733 (1987).

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
E10217	EnzChek® Phospholipase A ₂ Assay Kit *2 Plates*	1 kit
E10218	EnzChek® Phospholipase A ₂ Assay Kit *10 Plates*	1 kit
A10072	Red/Green BODIPY® PC-A2(1-O-(6-BODIPY®558/568-aminohexyl)-2-BODIPY®FL C5-Sn-glycero-3-phosphocholine)	100 µg

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