molecular probes®
by life technologies™

EnzChek® Phospholipase A_1 Assay Kit

Catalog numbers E10219, E10221

Table 1. Contents and storage information.

Material	E10219 (2-plates)	E10221 (10-plates)	Concentration	Storage	Stability
Phospholipase A_1 substrate, (PED-A1, Component A)	2 vials (70 µg each)	10 vials (70 µg each)	Not applicable	• ≤–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		• ≤–20°C • Desiccate	
Dioleoylphosphatidylglycerol (DOPG, Component C); MW = 797.04	800 µg	4 mg			
Phospholipase A_1 (Lecitase® Ultra, Component D)	50 Units	250 Units			
Dimethylsulfoxide (DMSO, Component E)	200 µL	1 mL		Desiccate	
5X Phospholipase A_1 reaction buffer, (Component F)	10 mL	50 mL	250 mM Tris-HCl, 0.7 M NaCl, 10 mM $CaCl_2$, pH 7.4	≤–20°C	

Number of assays: For Cat. no. E10219, sufficient material is supplied for 200 reactions in 96-well microplates at a volume of 100 µL per well as described in the following protocol or 800 reactions using low-volume 384-well microplates at a volume of ≤25 µL per well.
For Cat. no. E10221, sufficient material is supplied for 1000 reactions in 96-well microplates at a volume of 100 µL per well as described in the following protocol or 4000 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.

Introduction

The EnzChek® Phospholipase A_1 Assay Kit provides a simple, fluorometric method designed for continuous monitoring of phospholipase A_1 (PLA_1) activity. PLA_1 represents a family of enzymes that hydrolyze the *sn*-1 ester linkage of phospholipids and fatty acids.[1] The EnzChek® Phospholipase A_1 substrate (PED-A1) provides sensitive and continuous rapid, real-time monitoring of PLA_1 enzyme activities. (Figure 1).

The EnzChek® Phospholipase A_1 substrate (PED-A1) is specific for PLA_1 and is a dye-labeled glycerophosphoethanolamines with BODIPY® FL dye-labeled acyl chain at the *sn*-1 position and dinitrophenyl quencher-modified head group. Quenching efficiency is decreased by cleavage of the BODIPY® FL pentanoic acid substituent at the *sn*-1 position. The result is a PLA_1-dependent increase in BODIPY® FL fluorescence emission detected at approximately 515 nm. Specificity is imparted by the placement of the BODIPY® FL acyl chain in the *sn*-1 position and by incorporation of an acyl group with an enzymatic resistant (non-cleavable) ether linkage in the *sn*-2 position.

The EnzChek® Phospholipase A_1 Assay Kit can detect PLA_1 at 0.04 U/mL or lower (Figure 2, page 3). The assay is continuous and well suited for rapid and direct analysis of PLA_1 using automated instruments.

Figure 1. Fluorescence emission spectra of EnzChek® Phospholipase A_1 substrate incorporated in liposomes with addition of PLA_1 at room temperature.

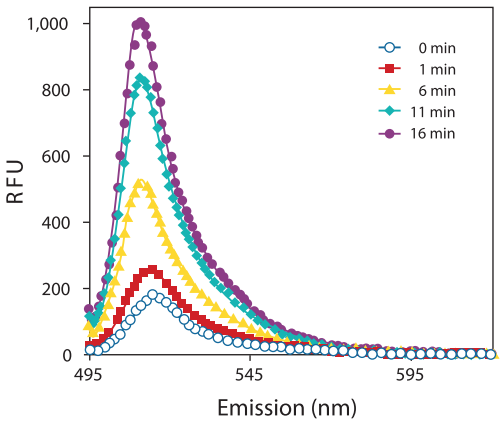
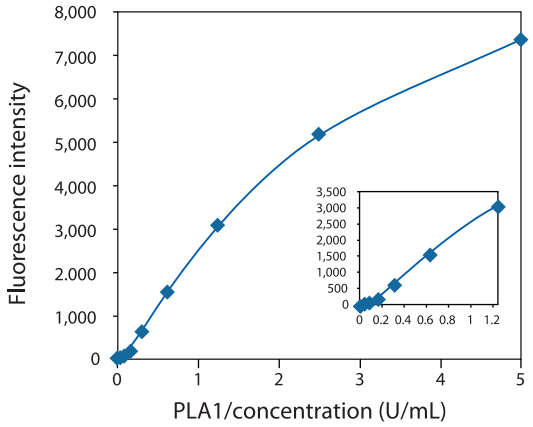


Figure 2. Plot of fluorescence emission intensities versus concentration of PLA_1 per well at 30 minutes, run at ambient temperature with liposomes. Fluorescence was measured exciting at 460 nm on a Spectra Max M5 (Molecular Devices). Background fluorescence determined for the no-PLA_1 enzyme control reaction has been subtracted.



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 µL 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 PLA_1 activity in samples.
- The following assay protocol is optimized for use with 96-well microplates using a 100 µL reaction volume per assay. For 384-well plates, adjust the reaction volumes accordingly to 25 µL 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 PLA_1 reaction buffer for optimal performance.

Caution

DMSO provided as a solvent in this kit, is known to facilitate the entry of organic molecules into tissues. Handle reagents containing DMSO using equipment and practices appropriate for the hazards posed by such materials. Dispose of reagents in compliance with all pertaining local regulations.

Preparing solutions

1.1 2 mM EnzChek® Phospholipase A_1 Substrate: Allow one vial of PLA_1 substrate (Component A) and DMSO (Component E) to warm to room temperature. Dissolve the contents of one vial of PLA_1 substrate in 40 µL DMSO. One vial of PLA_1 substrate is sufficient for approximately 100 assays with a final reaction volume of 100 µL per assay.

Store this stock solution frozen at ≤–20°C, **protected from light**.

1.2 1X EnzChek® PLA_1 Reaction Buffer: Add 4 mL of 5X PLA_1 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 µL 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. E10219) or 500 µL (Cat no. E10221) ethanol. Store solution at ≤–20°C.

1.4 10 mM DOPG (Dioleoylphosphatidylglycerol): Dissolve the contents of DOPG vial (Component C) in 100 µL (Cat. no. E10219) or 500 µL (Cat no. E10221) ethanol. Store solution at ≤–20°C.

1.5 500 Units/mL PLA_1 Stock Solution: Dissolve contents of the PLA_1 vial (Component D) in 100 µL (Cat. no. E10219, 2 plates) or 500 µL (Cat. no. E10221, 10 plates) of 1X PLA_1 reaction buffer prepared in step 1.2. Sufficient enzyme is supplied to prepare 100 (Cat. no. E10219) or 500 (Cat. no. E10221) positive control samples at 5 U/mL in an assay volume of 100 µL. Other sources of PLA_1 can be used as a positive control, but the sensitivity and dynamic range of the assay may be affected. Store stock solution at 4°C.

Experimental protocol

The following standard assay protocol is performed using a total volume of 100 µL per well. Samples and controls are mixed 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. Other volumes may be used; however, maintain the ratio of samples/controls to substrate at 1:1.

Assay protocol

2.1 Prepare a PLA_1 standard curve by diluting the appropriate amount of 500 Units/mL PLA_1 stock solution to 10 Units/mL in 1X PLA_1 reaction buffer to produce PLA_1 concentrations of 0–10 Units/mL, each in a volume of 50 µL. Final PLA_1 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 PLA_1 stock solution to 10 Units/mL in 1X PLA_1 reaction buffer. For a negative control, use 1X PLA_1 reaction buffer without PLA_1.

2.3 Dilute the PLA_1-containing samples in PLA_1 reaction buffer. You need 50 µL sample for each reaction. A variable dilution may be required depending on the total amount of PLA_1 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 2 mM PLA_1 substrate (from step 1.1).

2.6 Add 5 mL 1X PLA_1 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 30 minutes, **protected from light.** Because the assay is continuous (not terminated), fluorescence may be measured 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.

2.10 For each point, subtract the value derived from the no-PLA_1 control to correct for background fluorescence.

Troubleshooting

Problem	Cause	Solution
No response from the control enzyme	Low substrate concentration or substrate is contaminated	Substrate stock solution in DMSO appears yellow. 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_1 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_1 in the sample is highly active	Dilute sample until the response falls within the 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. Biochimie 89, 197 (2007).

Product List

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

Cat. no.	Product Name	Unit Size
E10219	EnzChek® Phospholipase A_1 Assay Kit *2 Plates*	1 kit
E10221	EnzChek® Phospholipase A_1 Assay Kit *10 Plates*	1 kit
Related Products		
A10070	PED-A1 N-{(6-(2,4-DNP)amino)hexanoyl}-1-(BODIPY®FL C5)-2-hexyl-*sn*-glycero-3-phosphoethanolamine *phospholipase A_1 selective substrate*	100 µg

Purchaser notification

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30 April 2014

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