Echelon Biosciences Inc.

Lysosomal Phospholipase A2 (LPLA2) Activity Assay

K-7000A (96 tests)

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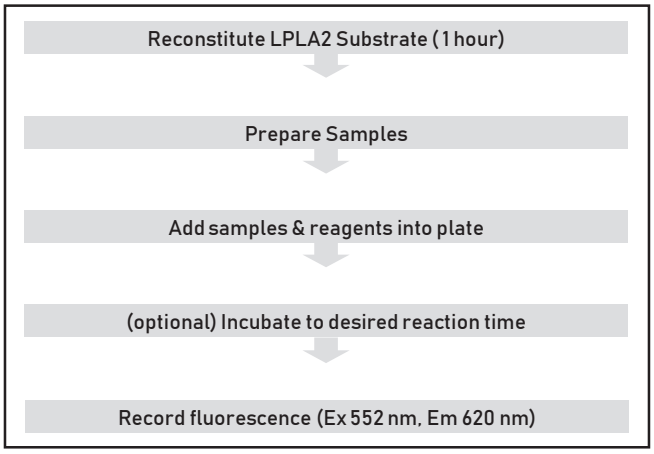
Description: The LPLA2 Activity Assay (K-7000A) is a homogenous assay designed to detect LPLA2 activity from both biological and purified sources of LPLA2.

Materials Provided

Catalog #	Description	Quantity
K-7001A	Human LPLA2 (200 ng)	1 vial
K-7002A	LPLA2 Substrate (650 nmol)	1 vial
K-7003A	5X Reaction Buffer (4 mL)	1 bottle
---	96-well Half-Area Black Plate	1 plate
---	Microtiter Plate Seals	2 seals

Quick Protocol



Additional Materials Provided by User

- Pipettes (capable of delivering between 5 and 1,000 µL solution with appropriate tips)
- Reagent grade water
- Plate shaker (optional)
- Microplate reader capable of reading with 552 nm excitation & 620 nm emission (see assay note #1)

Storage

Upon receipt, store assay kit at -20 °C. Opened and reconstituted reagents are less stable. Please see assay notes for additional information.

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Background

Human lysosomal phospholipase A2 (LPLA2) is responsible for normal lipid metabolism and is unique from other known PLA2s in that LPLA2 is only active in an acidic environment (around pH 4.5). Recent research suggests LPLA2 is related to drug-induced phospholipidosis (DIPL)[1]. DIPL is a condition of excessive accumulation of intracellular phospholipids triggered by long-term use of certain cationic amphiphilic drugs (CADs) such as fluoxetine (Prozac™, Sarafem) and Amiodarone. DIPL progresses through tissue inflammation and organ damage. If not caught in time, DIPL can result in death. The FDA has determined that DIPL is a serious drug safety issue.

Assay Design

Echelon's LPLA2 Activity Assay (K-7000A) is a homogenous assay designed to detect LPLA2 activity from both biological and purified source of LPLA2. The assay uses a quenched substrate which fluoresces when hydrolyzed by LPLA2 (see Figure 1). The sn-1 position of the quenched substrate is protected by an amine group to eliminate non-specific turnover by phospholipase A1. The acidic assay conditions further improve selectivity of LPLA2 for hydrolyzing the quenched substrate. This direct biochemical approach provides a specific quantitative measurement in a robust and simple-to-use in vitro plate-based assay for quick LPLA2 activity measurement.

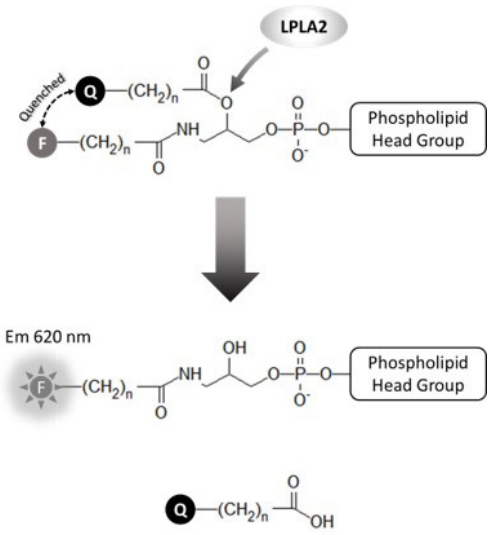
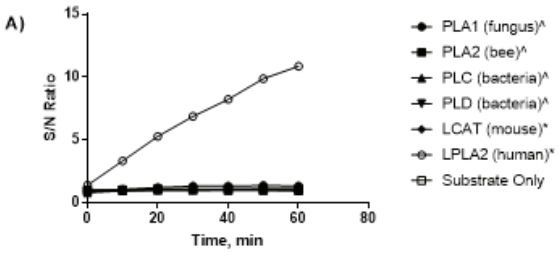


Figure 1. Assay Design
Q = qunecher, F = Fluorophore

Specificity

No significant cross reactivity observed against any phospholipases and structurally related enzymes at 100 times higher concentration than the tested LPLA2 concentration (Figure 2). As it is impossible to run a complete interference test, the possibility of interference cannot be excluded.



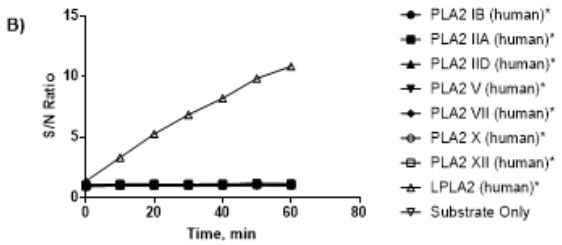


Figure 2. Assay Cross Reactivity. A) Phospholipases and lecithin–cholesterol acyltransferase (LCAT). B) Phospholipase A2 (PLA2). LPLA2 and other enzymes were tested at 20 ng/mL and 2 µg/mL respectively. ^ - purified enzymes. * - recombinant enzymes.

Sample Type

This kit detects human or animal LPLA2 activity in serum, plasma and urine as well as purified enzymes. LPLA2 activity differences were successfully detected between wildtype (WT) and LPLA2 (KO) mice plasma (Figure 3)[2].

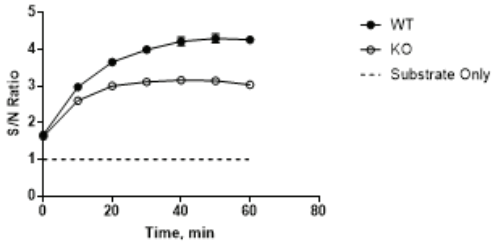


Figure 3. Mouse Plasma LPLA2 Activity

Sample Volume

For duplicate data points 30 µL of each sample is required.

Assay Performance

For best results, please follow the protocols provided. Not following the instructions may result in suboptimal performance of the kit and failure to produce accurate data.

Assay Notes

1. The rehydrated LPLA2 Substrate solution should be dark purple in color and translucent without debris. Additional vertexing and 3-5 minutes ice bath sonication can be applied if debris is seen. However, variation of LPLA2 rehydration will affect assay-to-assay producibility. Store rehydrated LPLA2 Substrate at -20 °C. Limit freeze defrost cycles.
2. Store the remaining Human LPLA2 (K-7001A) at -20 °C. The Human LPLA2 enzyme is stable for at least 1 month when store at -20 °C with limited freeze-defrost cycles.
3. The recommended sample dilutions are listed in the table. Due to variability between samples, sample dilution optimization is highly recommended before running a panel of samples. Sample final concentration >20% is not recommended.

Sample Type	Recommended Concentration	
	Reaction (1X)	Sample (5X)
Serum/Plasma	5-10%	25-50%
Urine	10-20%	50%-100%

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4. Ensure samples are free of debris. If needs, centrifuge samples at 10,000 x g for 5 minutes at 4 °C to remove debris. Carefully pipette superannuant to use as sample.
5. The LPLA2 Activity Assay is designed to run at RT. If sample LPLA2 activity is low, assay can be run at 37 °C to improve the assay sensitivity. Assays performed under controlled temperature environment will improve day-to-day reproducibility.
6. Due to the narrow excitation and emission wavelength of the red fluorophore, filter-based microplate reader with 552 nm excitation filter and 620 nm emission filter is recommended. If only monochromator microplate reader is available, lower excitation and higher emission wavelength should be used. LPLA2 Activity Assay signal has been successfully detected with monochromator microplate reader using excitation at 515 nm and emission at 695 nm. However, loss of assay sensitivity and increase of assay coefficient of variation were observed.
7. Due to instrument variation, microplate reader optimization using 20 ng/mL of the provided Human LPLA2 (K-7001A) as a positive control and reagent grade water as a negative control is highly recommended before running the full panel of samples.

Assay Protocol

Please read the entire Assay Protocol and the Assay Notes section before beginning the assay. This protocol has been developed for duplicate reaction points. If other replicates are preferred, adjust the protocol accordingly.

1. Place Human LPLA2 (K-7001A) on ice. Equilibrate other reagents to room temperature.
2. Rehydrate the LPLA2 Substrate (K-7002A, 650 nmol) with 1.3 mL reagent grade water for 500 µM stock solution. Incubate at RT for 1 hour without vortex. After rehydration, vortex for 1 minute to fully rehydrate the substrate. Insufficient substrate rehydration will significantly affect the assay performance. See assay notes for additional information
3. Add 20 µL reagent grade water to the 200 ng Human LPLA2 (K-7001A) for 10 µg/mL stock. Pipette up and down to mix. Do not vortex. Dilute 3 µL of the 10 µg/mL stock with 297 µL reagent grade water for 5X stock at 100 ng/mL. Pipette up and down to mix. Do not vortex. Keep on ice until use.
4. Prepare samples at 5X of the desired testing concentration in water. Keep on ice. See assay notes for additional information.
5. Start reactions by sequentially adding the followings in duplicates:
   a. 24 µL of the reagent grade water
   b. 12 µL of the 5X Reaction Buffer (K-7003A)
   c. 12 µL of the rehydrated LPLA2 Substrate (K-7002A)
   d. 12 µL of the 5X Human LPLA2 (positive control), the reagent grade water (negative control) or the 5X samples
   e. Tap or shake plate to mix.
6. (Optional) If end-point assay is preferred, cover plate with the provided microtiter plate seal. Protect from light. Incubate plate for desired time before proceeding to step 7.
7. Record fluorescence kinetically with the desired intervals using 552 nm excitation and 620 nm emission. See assay notes for additional information.

Data Analysis

Plot the recorded relative fluorescence units (RFU) as y-axis and time as x-axis to generate sample reaction progress curves. Use the slope of the linear range of the reaction progress curves to obtain samples' LPLA2 activity at RFU/min. A typical reaction progress curve of the Human LPLA2 (K-7001A) is shown at Figure 4.

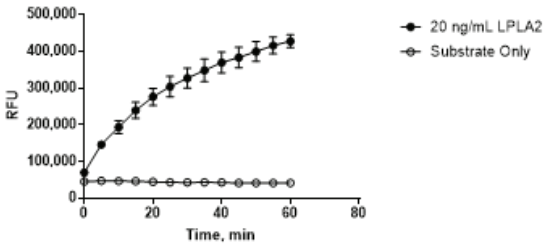

Figure 4. Human LPLA2 Reaction Progress Curve

References (Assay Background)

1. Abe, A.; Hiraoka, M.; Shayman, J. A.; Ohguro, H., A fluorometric assay for lysosomal phospholipase A2 activity using fluorescence-labeled truncated oxidized phospholipid. Anal Biochem 2018, 549, 164-170.
2. Hiraoka, M.; Abe, A.; Lu, Y.; Yang, K.; Han, X.; Gross, R. W.; Shayman, J. A., Lysosomal phospholipase A2 and phospholipidosis. Mol Cell Biol 2006, 26 (16), 6139-48.

Related Products

Products	Catalog Number
Enzyme	
Human LPLA2	E-7000
Antibody	
Purified Anti-LPLA2	Z-PLPLA2
Assay	
LPLA2 Inhibitor Screen	K-7000I
LPLA2 ELISA	Coming Soon

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