

Lysophosphatidic Acid (LPA) Assay Kit II

K-2800S (96 tests)

Support: echelon@echelon-inc.com

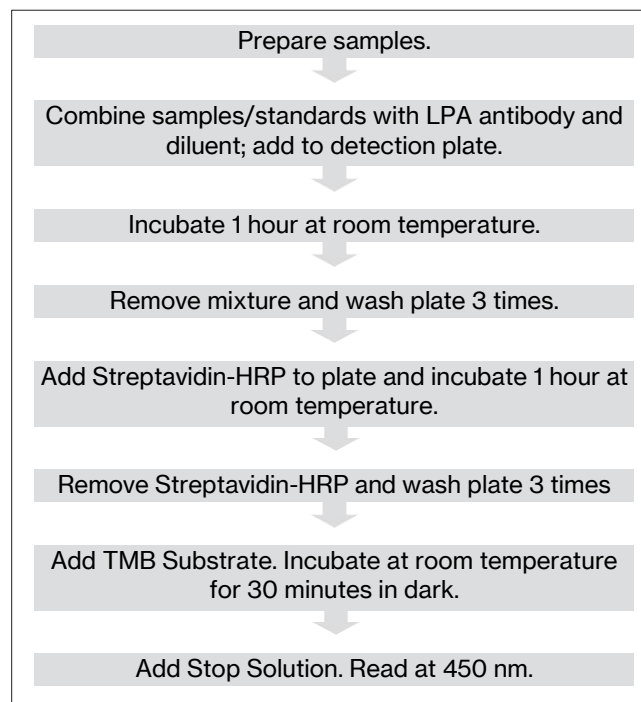
Description: The Lysophosphatidic Acid ELISA (LPA Assay Kit II) is a sensitive and robust method for quantification of LPA in Plasma, Serum, and Tissue Homogenate.

Storage: upon receipt, store part 1 (kit box) at 4 °C and part 2 at -80 °C. The LPA standard is not stable at temperatures > -70 °C. Under proper storage, this product is stable 6 months from date of receipt. Opened and reconstituted solutions are less stable.

Materials Provided

Catalog #	Description	Quantity
K-2801S	Biotinylated Anti-LPA Antibody	< 100 µL
K-2805L	Lysophosphatidic Acid (C18:3) Standard	238 µg
K-2807S	LPA Detection Plate	1 Plate
K-SEC3h	Streptavidin HRP	80 µL
K-PBST4	Sample Diluent	10 mL
K-PBST2	10X PBS-T Buffer	20 mL
K-TMB1	TMB Substrate	12 mL
K-STOPt	1N Sulfuric Acid (H2SO4)	8 mL
---	Microtiter plate seal	2 Seals

Quick Protocol



Additional materials provided by user

- pipettes (capable of delivering between 5 and 1,000 µL with appropriate tips)
- multichannel pipettes
- absorbance microplate reader capable of reading at 450 nm

Echelon biosciences products are sold for research and development purposes only and are not to be incorporated into products for resale without written permission from echelon biosciences. This kit is covered by a u.s. patent and includes a limited, non-transferable license under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. For inquiries email echelon@echelon-inc.com

Background

Lysophosphatidic Acid (LPA) is a serum-derived phospholipid involved in diverse cellular processes such as cell proliferation, chemotaxis, platelet aggregation, wound healing, angiogenesis, tumor invasion, and smooth muscle contraction. Recent research indicates LPA may play a significant role in the pathophysiology of cancer and may be used as a biomarker for ovarian cancer.

Assay Design

The Lysophosphatidic Acid (LPA) Assay Kit II is an enzyme-linked immunoassay designed for the in vitro measurement of LPA in biological samples. The assay is a competitive ELISA in which the colorimetric signal is inversely proportional to the amount of LPA present in the sample (increased LPA = reduced assay signal). Briefly, the samples are pre-mixed with the biotinylated anti-LPA antibody and a sample diluent before the mixtures are added to the LPA coated detection plate for competitive binding. Streptavidin-HRP and colorimetric detection are used to detect the amount of biotinylated anti-LPA bound to the plate. The concentration of LPA in the sample is determined using a standard curve of known amounts of LPA. This assay is read at 450 nm and requires 3 hours to run.

Assay Range: 0.064 μ M to 1,000 μ M

Sensitivity

The limit of detection (LOD), defined as the lowest amount of LPA in a sample detectable from zero, was calculated at 0.12 μ M. The limit of quantification (LOQ) or the lowest concentration detected within the linear range of the curve was calculated at 0.024 μ M. The LOD and LOQ values are from an average of 4 experiments.

Specificity

No significant cross reactivity was observed against any lipid tested. The lipids we selected for testing were related in structure and signaling pathway. For a complete list of the lipids tested please contact customer service. As it is impossible for us to run a complete cross-reactivity and interference test on the assay, cross reaction may still exist.

Sample Type: This kit detects LPA from human or animal in serum, plasma and tissue homogenate.

Sample Volume: 20 μ L/ sample is needed for duplicate data points.

Assay Performance

For the 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. Ensure samples are free from debris before addition to plate.
2. Ensure all reagents are warmed to room temperature before use. Failing to do so might result in edge effects and increased CV's.
3. Samples/standards: Antibody: Sample Diluent mixing ratio, in step 5, is 1:9:10. Volumes can be adjusted. Sample

Diluent must be added last. Mixture is mixed by vortex for 1-2 seconds to achieve the optimal assay reproducibility.

4. When analyzing biologic samples, we advise running a known normal (low) LPA sample and a disease (high) LPA sample in conjunction with your unknown samples. These will serve as positive and negative controls to distinguish between normal healthy samples and disease samples.
5. The antibody used in this assay has varied binding affinity to different LPA species. The order of the antibody affinity to different LPA species are C18:3 > C18:2 > Mixed LPA > C20:4 > C14:0 > C18:1 > C16:0 > C18:0. Mixed LPA contains LPA C16:0, C18:0, C18:1, C18:2 and C20:4 at 2.8:1.5:2.6:3 concentration ratios.
6. This assay is optimized for human serum and plasma. The assay can be used for LPA detection in animal samples, but this application has not been optimized. Sample dilution may need to be optimized and is highly recommended before running a panel of samples.
7. The LPA Assay Kit II has been optimized for the detection of LPA in serum and plasma samples. Sample optimization is highly recommended for tissue homogenate samples.

Protocol for LPA Detection

Please read this entire section and the assay notes section before beginning the assay. This protocol has been developed for duplicate reaction points. If singlet or triplicate points are required, the protocol will need to be adjusted accordingly. To begin, prepare samples (see support protocols) and place the Streptavidin HRP (K-SEC3h), and Biotinylated Anti-LPA Antibody (K-2801S) on ice. Allow the remaining kit components to warm to room temperature before use.

1. Prepare 1X PBST by diluting the bottle of 10X PBS-T Buffer (K-PBST2) with 180 mL deionized water. Store prepared 1X PBST at room temperature.
2. Reconstitute the LPA Standard (K-2805L)
 - a. For serum or plasma samples, reconstitute the LPA Standard (K-2805L) with 50 μ L of 1X PBST (step 1) for 1,000 μ M solution. Vortex to mix and keep at room temperature. The reconstituted LPA standard can handle at least one freeze-defrost cycle and is stable for 6 months at < -20°C.
 - b. For tissue homogenate samples, reconstitute the LPA Standard (K-2805L) with 50 μ L of tissue homogenate buffer (see buffer recipe in support protocols) for 1,000 μ M solution. Vortex to mix. Keep at room temperature. If samples have been prepared in two different buffers, then a separate standard curve will need to be made for each sample buffer used. An extra LPA standard can be purchased under cat# K-2805L. The standards stability in homogenate buffer is unknown.
3. Vortex reconstituted LPA standard (step 2). Prepare the 7 LPA standard dilutions in 1X PBST (step 1) or homogenate buffer as described in Table 1.
4. Add 7 mL 1X PBST (step 1) directly into the Biotinylated

Anti-LPA Antibody bottle (K-2801S). Mix well. Place at room temperature and immediately proceed to the next step. This reagent is not stable at working concentration and should be prepared immediately before use. The diluted antibody can handle at least one freeze/defrost cycle and is stable for 7 days storage at -20 °C. A change in the assays signal may be observed.

5. Combine the 7 LPA standards (step 3), or samples, with the diluted antibody (step 4) and Sample Diluent (K-PBST4) in 1.5 mL polypropylene tubes in the following order. If desired, a 96-well incubation plate can be used instead of the 1.5 mL polypropylene tubes.
 - a. First, add 135 μ L of the diluted antibody (step 4) into each tube. For negative control, add 135 μ L of 1X PBST (step 1).
 - b. Second, add 15 μ L of the LPA standards (step 3) or prepared samples into appropriate tubes. For positive and negative controls, add 15 μ L of the solution used to dilute the standards. This should be 1X PBST (step 1) and/ or homogenate buffer.
 - c. Third, add 150 μ L of Sample Diluent (K-PBST4) to all tubes.
 - i. Ensure pipette tip does not contact the sample/antibody mixture to avoid cross contamination between samples.
 - ii. If precipitation is present in the Sample Diluent (K-PBST4), vortex vigorously until precipitate is dissolved.
 - d. Vortex each tube for 1-2 seconds to mix.
6. Incubate tubes for 15-20 minutes at room temperature.
7. Remove the LPA Detection Plate (K-2807S) from plastic bag. Transfer 100 μ L/well of the prepared standards, controls, and samples (step 5) according the “Suggested LPA Detection Plate Layout”, see Table 2 below. For best results, prime the pipette 3-6 times before transferring. Cover the plate with a plate seal and incubate at room temperature for 1 hour.
8. After incubation, discard solutions from the LPA Detection Plate and wash with 200 μ L/well of the 1X PBST (step 1) three times.
9. Dilute 60 μ L of the Streptavidin HRP (K-SEC3h) with 12 mL of the 1X PBST (step 1). Mix well. If you are running experiments over multiple days, only prepare what is needed for the day. Prepare immediately before use. The stock Streptavidin HRP (K-SEC3h) can withstand 3 freeze-defrost cycles and should be stored at -20°C to -80°C between uses.
10. Remove wash buffer from plate. Add 100 μ L/well of the diluted Streptavidin HRP solution (step 8) to the LPA Detection Plate. Cover with a new plate seal and incubate at room temperature for 1 hour.
11. After incubation, discard solutions from the LPA Detection Plate and wash with 200 μ L/well of the 1X PBST (step 1) three times. Ensure the wash buffer is removed before proceeding to the next step.
12. Add 100 μ L/well of the TMB Substrate (K-TMB1) and incubate for 30 minutes in a dark location. Then add 50 μ L/well 1N Sulfuric Acid (K-STOPT) to the Microtiter Plate to stop the reaction. Read Microtiter Plate absorbance at 450 nm.

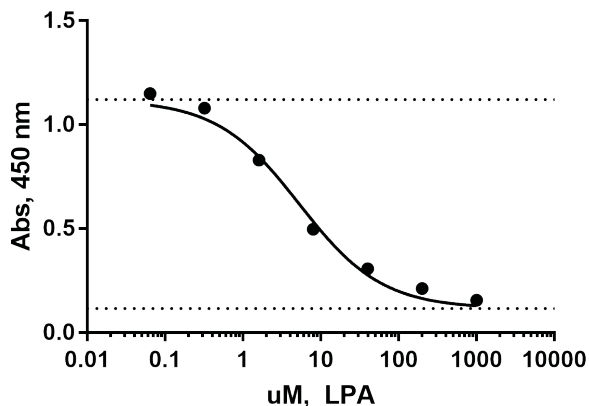
Table 1, LPA Standards

LPA Concentration	Amount of 1,000 μ M LPA Stock (Step 3) or Previous Dilution	Amount of 1X PBST or homogenate buffer
1000 μ M	45 μ L of 1000 μ M LPA Solution	None
200 μ M	5 μ L of 1000 μ M LPA Solution	20 μ L
40 μ M	5 μ L of 200 μ M LPA Solution	20 μ L
8 μ M	5 μ L of 40 μ M LPA Solution	20 μ L
1.6 μ M	5 μ L of 8 μ M LPA Solution	20 μ L
0.32 μ M	5 μ L of 1.6 μ M LPA Solution	20 μ L
0.064 μ M	5 μ L of 0.32 μ M LPA Solution	20 μ L
0 μ M (Pos. Ctrl)	0 μ L	25 μ L

Table 2, Suggested LPA Detection Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	1000 μ M LPA	1000 μ M LPA	Sample #1	Sample #1	Sample #8	Sample #8	Sample #16	Sample #16	Sample #24	Sample #24	Sample #32	Sample #32
B	200 μ M LPA	200 μ M LPA	Sample #2	Sample #2	Sample #9	Sample #9	Sample #17	Sample #17	Sample #25	Sample #25	Sample #33	Sample #33
C	40 μ M LPA	40 μ M LPA	Sample #3	Sample #3	Sample #10	Sample #10	Sample #18	Sample #18	Sample #26	Sample #26	Sample #34	Sample #34
D	8 μ M LPA	8 μ M LPA	Sample #4	Sample #4	Sample #11	Sample #11	Sample #19	Sample #19	Sample #27	Sample #27	Sample #35	Sample #35
E	1.6 μ M LPA	1.6 μ M LPA	Sample #5	Sample #5	Sample #12	Sample #12	Sample #20	Sample #20	Sample #28	Sample #28	Sample #36	Sample #36
F	0.32 μ M LPA	0.32 μ M LPA	Sample #6	Sample #6	Sample #13	Sample #13	Sample #21	Sample #21	Sample #29	Sample #29	Sample #37	Sample #37
G	0.064 μ M LPA	0.064 μ M LPA	Sample #7	Sample #7	Sample #14	Sample #14	Sample #22	Sample #22	Sample #30	Sample #30	Sample #38	Sample #38
H	Pos. Ctrl.	Pos. Ctrl.	Neg. Ctrl.	Neg. Ctrl.	Sample #15	Sample #15	Sample #23	Sample #23	Sample #31	Sample #31	Sample #39	Sample #39

Quantification of Samples



Generate a best fit curve for the LPA standards to interpolate relative sample values. The example LPA standard curve, above, shows a 7-point LPA curve that was generated using non-linear regression analysis with Graphpad Software. A semi log [Sigmoidal dose-response (variable slope)] (4 parameter) analysis was utilized. For best results, constrain the standard curve top & bottom using the positive and negative controls. If samples have been diluted, the dilution factor must be multiplied back into the concentration calculated.

Support Protocols

Preparation of Plasma

Materials and Equipment

- Human blood sample
- Vacutainer tubes containing anticoagulant
- Serological pipettes of appropriate volumes (sterile), centrifuge tubes, cryovials
- Benchtop centrifuge (NOT refrigerated) with swing-out rotor and appropriate carriers

Procedure

1. Draw blood into vacutainer tube(s) containing anticoagulant following local standard operating procedures (this may vary depending on manufacturer). Be sure to draw the full volume to ensure the correct blood-to anticoagulant ratio.
2. Invert vacutainer tubes carefully 10 times to mix blood and anticoagulant and store at room temperature until centrifugation.
3. Samples should undergo centrifugation immediately. This should be carried out for a minimum of 10 minutes at 1000-2000 RCF (generally 1300 RCF) at room temperature (refer to speeds and times recommended by manufacturer). Do not use brake to stop centrifuge.
4. This will give three layers: (from top to bottom) plasma, leukocytes (buffy coat), erythrocytes. Carefully aspirate the supernatant (plasma) at room temperature and transfer to a centrifuge tube. Take care not to disrupt the cell layer or transfer any cells.
5. Inspect plasma for turbidity. Turbid samples should be

centrifuged and aspirated again to remove remaining insoluble matter.

6. Aliquot plasma into cryovials and store at -80°C . Ensure that the cryovials are adequately labeled with the relevant information, including details of additives present in the blood.

Preparation of Serum

Materials and Equipment

- Human blood sample
- Vacutainer tubes (containing either no additive or a clot activator)
- Serological pipettes of appropriate volumes (sterile), centrifuge tubes, cryovials
- Benchtop centrifuge (NOT refrigerated) with swing-out rotor and appropriate carriers

Procedure

1. Draw whole blood into vacutainer tube(s) containing no anticoagulant following local standard operating procedures. Draw approximately $2\frac{1}{2}$ times the volume needed for use e.g. 10 mL blood for 4 mL serum.
2. Incubate in an upright position at room temperature for 30-45 min (no longer than 60 min) to allow clotting. If using a clot activator tube, invert carefully 5-6 times to mix clot activator and blood before incubation.
3. Centrifuge for 15 minutes at manufacturer's recommended speed (usually 1,000-2,000 RCF). Do not use brake to stop centrifuge.
4. Carefully aspirate the supernatant (serum) at room temperature and transfer into a centrifuge tube, taking care not to disturb the cell layer or transfer any cells. Use a clean pipette for each tube. Inspect the serum for turbidity. Turbid samples should be centrifuged and aspirated again to remove remaining insoluble matter.
5. Aliquot into cryovials and store at -80°C . Ensure that the cryovials are adequately labeled with the relevant information, including details of additives present in the blood.

Preparation of Tissue Homogenate

For tissue homogenate and cell culture samples we recommend $>30\ \mu\text{g}$ of total protein/well. It is highly suggested to determine the amount of total protein/well needed for your sample before you run your experiments. Concentration of the sample may be required.

Procedure

1. Homogenized tissues were tested in the following methods.
 - a. By sonication in the following homogenization buffer: 20 mM Tris-HCl, pH 7.4; 20% glycerol; 1 mM β -mercaptoethanol; 1 mM EDTA; 1 mM Naorthovanadate; 15 mM NaF; 1 mM PMSF; protease inhibitor cocktail (Sigma); 0.5 mM deoxy-pyridoxine; 40 mM β -glycerophosphate. Centrifuge 5 minutes at $5000 \times g$, $2 - 8^{\circ}\text{C}$. The supernatant is then removed and assayed.
 - b. By freeze-defrost cycles in 1X PBS: Rinse tissue

in 1X PBS and store overnight in 1X PBS. Perform two freeze-thaw cycles. Centrifuge 5 minutes at 5000 x g, 2 - 8°C. The supernatant is then removed and assayed.

- Total protein concentration should be measured. Dilute samples with the homogenate buffer to the same protein concentration before being added to the assay.
- The homogenates can be stored frozen at -80°C until analysis. Avoid repeat freeze-thaw cycles. Centrifuge the sample again after thawing and before adding to the assay.

References

- Michalczyk A, Budkowska M, Dogowska B, Chlubek D, Safranow K. Lysophosphatidic acid plasma concentrations in healthy subjects: circadian rhythm and associations with demographic, anthropometric and biochemical parameters. *Lipids in Health and Disease*. 2017;16:140.
- Caraher EJ, Kwon S, Haider SH, Crowley G, Lee A, Ebrahim M, et al. Receptor for advanced glycation end-products and World Trade Center particulate induced lung function loss: A case-cohort study and murine model of acute particulate exposure. *PLoS ONE*. 2017;12(9)
- McArthur S, Gobbetti T, KÅsters DHM, Reutelingsperger CP, Flower RJ, Perretti M. Definition of a Novel Pathway Centered on Lysophosphatidic Acid To Recruit Monocytes during the Resolution Phase of Tissue Inflammation. *J Immunol*. 2015.
- Tsukiji J, Cho SJ, Echevarria GC, Kwon S, Joseph P, Schenck EJ, et al. Lysophosphatidic acid and apolipoprotein A1 predict increased risk of developing World Trade Center-lung injury: a nested case-control study. *Biomarkers*. 2014;19(2):159-65.
- Tang N, Zhao Y, Feng R, Liu Y, Wang S, Wei W, et al. Lysophosphatidic acid accelerates lung fibrosis by inducing differentiation of mesenchymal stem cells into myofibroblasts. 2014.
- Simo KA, Niemeyer DJ, Hanna EM, Swet JH, Thompson KJ, Sindram D, et al. Altered lysophosphatidic acid (LPA) receptor expression during hepatic regeneration in a mouse model of partial hepatectomy. *HPB (Oxford)*. 2014;16(6):534-42.
- Cong C, Mao L, Zhang Y, Zhao Z, Xu X, Zhao J. Regulation of silicosis formation by lysophosphatidic acid and its receptors. *Exp Lung Res*. 2014;40(7):317-26.
- Balood M, Zahednasab H, Siroos B, Mesbah-Namin SA, Torbati S, Harirchian MH. Elevated serum levels of lysophosphatidic acid in patients with multiple sclerosis. *Hum Immunol*.
- Abu El-Asrar AM, Nawaz MI, Mohammad G, Siddiquei MM, Alam K, Mousa A, et al. Expression of bioactive lysophospholipids and processing enzymes in the vitreous from patients with proliferative diabetic retinopathy. *Lipids Health Dis*. 2014;13:187.
- Skill N, Wu J, Xu Y, Zhao Z, Maluccio M. Lysophosphatidic acid aberrancies and hepatocellular carcinoma: studies in the MDR2 gene knockout mouse. *Cancer Invest*. 2013;31(2):145-55.
- Chattopadhyay A, Navab M, Hough G, Gao F, Meriwether D, Grijalva V, et al. A Novel Approach to Oral ApoA-I Mimetic Therapy. *J Lipid Res*. 2013.
- Goncalves I, Edsfeldt A, Ko NY, Grufman H, Berg K, Bjorkbacka H, et al. Evidence supporting a key role of Lp-PLA2-generated lysophosphatidylcholine in human atherosclerotic plaque inflammation. *Arterioscler Thromb Vasc Biol*. 2012;32(6):1505-12.
- G. Linkous A, E. Hallahan D, M. Yazlovitskaya E. Autotaxin and Lysophosphatidic Acid Receptors as Novel Targets for the Radiosensitization of Tumor Vasculature in Non-Small Cell Lung Cancer. 2012.
- Badri L, Lama VN. Lysophosphatidic acid induces migration of human lung-resident mesenchymal stem cells through the beta-catenin pathway. *Stem cells (Dayton, Ohio)*. 2012;30(9):2010-9.
- Skill NJ, Scott RE, Wu J, Maluccio MA. Hepatocellular carcinoma associated lipid metabolism reprogramming. *J Surg Res*. 2011;169(1):51-6.
- Mazzocca A, Dituri F, Lupo L, Quaranta M, Antonaci S, Giannelli G. Tumor-secreted lysophosphatidic acid accelerates HCC progression by promoting differentiation of peritumoral fibroblasts in myofibroblasts. *Hepatology (Baltimore, Md.)* 2011.
- Song HY, Lee MJ, Kim MY, Kim KH, Lee IH, Shin SH, et al. Lysophosphatidic acid mediates migration of human mesenchymal stem cells stimulated by synovial fluid of patients with rheumatoid arthritis. *Biochim Biophys Acta*. 2010;1801(1):23-30.

Related Products

Products	Catalog Number	Products	Catalog Number
Assays, Antibodies and Enzymes		Lipids	
Autotaxin Enzyme, active	E-4000	LPA Biotin	L-012B
Autotaxin Activity Assay	K-4100	LPA C18:1, LPA C18:2, LPA C18:3	L-0181, L-0182, L-0183
Autotaxin Inhibitor Screening Kits	K-4200, K-4200HTS	Ether-linked LPA	L-0186
Autotaxin ELISA	K-5600	LPA C20:0, LPA C20:4	L-0200, L-0204
Anti-LPA Mouse Monoclonal Antibody	Z-P200	Inhibitors	
Fluorescent ATX Substrates		XY-14 (Lipid Phosphate Phosphatase 1 (LPP1) inhibitor)	L-9218
FS-3 (lysoPLD Substrate)	L-2000	LPA3 (edg 7) selective ligands	L-9118, L-9318, L-9418, L-9518
ATX-Red AR-2	L-2010	BrP-LPA	L-7416
		ATX Inhibitors	B-0701, B-0702, L-3223, L-3282, L-7118, L-7218