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Technical Data Sheet

For research use only
Not intended or approved for diagnostic or therapeutic use.

The LPA Assay Kit was co-developed using the proprietary monoclonal antibody of Lpath Inc. (San Diego, CA. www.lpath.com).

Product Name: Lysophophatidic Acid Assay Kit (96-well ELISA)

Product Number: K-2800

Kit Components:

Part #	Description
K-2801	**Anti-LPA Antibody**
K-2802	**Anti-LPA Antibody Diluent**
K-2803	**Block Solution**
K-2804	**Delipidized Human Sera**
K-2805	**Lysophophatidic Acid (LPA) Standard**
K-2806	**Anti-mouse goat IgG - HRP**
K-2807	**Coated Microtiter plate** (sealed in plastic bag)
K-TMB1	**TMB Substrate**
K-STOPt	**1N Sulfuric Acid (H_2SO_4)**
---	**Microtiter plate seal**
---	**PBS Tablets**
---	**Incubation plate** (yellow)

Each kit provides sufficient reagents for 96 samples assay (including standards).

Researcher must provide:

Absorbance microtiter plate reader capable of reading at 450 nm.
Pipettes (20 µL, 200 µL, and 1,000 µL)

Storage:

Upon receipt, the kit should be stored at –20 °C protected from light. The TMB substrate, K-TMB1, needs to be removed from the kit and stored at 4 ºC. Under proper storage conditions, the kit components should remain stable for at least 6 months from date of receipt. Allow the reagents to warm to room temperature before opening vials.

Health Hazard Data:

The toxicity effects of delipidized human sera have not been determined. The kit contains delipidized human serum and should be handled as potentially bio-hazardous materials. The serum was derived from blood donors tested individually and shown by FDA approved methods to be negative for antibodies to Human Immunodeficiency Virus 1/2 (HIV) and Hepatitis C Virus (HCV), non-reactive to Human Immunodeficiency Virus 1 (HIV1), Hepatitis B surface antigen (HBsAg) and Sexually Transmitted Diseases (RPR). Since no test method can offer complete assurance that HIV, Hepatitis B and C Virus or other infectious agents are absent, these products should be handled by trained researchers using safe laboratory techniques and approved procedures.

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. (*several literature citations indicate plasma LPA levels are elevated in ovarian cancer patients). The Echelon LPA assay is a sensitive and robust method for the quantification of LPA in a 96-well plate format. ***The LPA Assay Kit was co-developed using the proprietary monoclonal antibody of Lpath Inc. (San Diego, CA. www.lpath.com).***

Assay Procedure:

1. Bring the assay kit to room temperature before use.

2. Remove coated microtiter plate (Nunc, Maxisorp) from plastic bag.

3. Block each well of microtiter plate by adding 150 µL of block solution per well and incubate at room temperature for 1 hour (place microtiter plate seal on plate).

4. Prepare PBS (washing buffer) by dissolving *each* PBS tablet in 200mL deionized water.

5. Wash plate with PBS four times (4X) and on the final wash incubate the plate for 2 minutes before removing the wash solution. Ensure all wash buffer is removed before progressing to the next step.

6. Vortex LPA standard vial and prepare standard dilutions of LPA Standard as follows (use standard 1.5mL polypropylene tubes):

µM LPA [final]	µl of 200 µM stock or previous dilution	µl diluent (Delipidized Human Sera)
5 µM	15 µl 200µM LPA Stock Soln.	585 µl
2.5 µM	300 µl 5 µM Solution	300 µl
1.25 µM	300 µl 2.5 µM Solution	300 µl
0.63 µM	300 µl 1.25 µM Solution	300 µl
0.31 µM	300 µl 0.63 µM Solution	300 µl
0.16 µM	300 µl 0.31 µM Solution	300 µl
0.08 µM	300 µl 0.16 µM Solution	300 µl
0 µM	-	300 µl

7. Dilute anti-LPA antibody by adding 4 mL anti-LPA antibody diluent to the bottle containing the anti-LPA antibody and mix well.

8. Dilute samples in delipidized human sera (suggested dilution is 1:10, 30 µL sample: 270 µL delipidized human sera). Combine samples and standards with diluted anti-LPA antibody and mix in the incubation plate (yellow) before transferring to the coated, blocked, and washed Microtiter plate (Nunc, Maxisorp). ***If replicates other than duplicates are preferred, add the diluted antibody to the diluted sample 1:4.***
    a. Add 75µL of diluted antibody to each well of the mixing plate (see plate layout below).
    b. Add 225µL of standards or diluted samples to each well of the mixing plate (see plate layout below).

Suggested Mixing Plate Layout (yellow)

	1	2	3	4	5	6	7	8	9	10	11	12
A	5 µM	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33	*-Empty-*	*-Empty-*	*-Empty-*	*-Empty-*	*-Empty-*	*-Empty-*
B	2.5 µM	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34	*-Empty-*	*-Empty-*	*-Empty-*	*-Empty-*	*-Empty-*	*-Empty-*
C	1.25 µM	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35	*-Empty-*	*-Empty-*	*-Empty-*	*-Empty-*	*-Empty-*	*-Empty-*
D	0.63 µM	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36	*-Empty-*	*-Empty-*	*-Empty-*	*-Empty-*	*-Empty-*	*-Empty-*
E	0.31 µM	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37	*-Empty-*	*-Empty-*	*-Empty-*	*-Empty-*	*-Empty-*	*-Empty-*
F	0.16 µM	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38	*-Empty-*	*-Empty-*	*-Empty-*	*-Empty-*	*-Empty-*	*-Empty-*
G	0.08 µM	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39	*-Empty-*	*-Empty-*	*-Empty-*	*-Empty-*	*-Empty-*	*-Empty-*
H	0 µM	Sample 8	Sample 16	Sample 24	Sample 32	Sample 40	*-Empty-*	*-Empty-*	*-Empty-*	*-Empty-*	*-Empty-*	*-Empty-*

9. Place incubation plate on plate shaker for 30 seconds; or carefully tap the plate to mix.

10. Add 100µL of mixture from the mixing plate to each well according to plate layout of the coated, blocked, and washed microtiter plate (Nunc, Maxisorp).

    Suggested duplicates sample microtiter plate layout (Nunc, Maxisorp). ***Samples can be run in single, duplicate, or triplicate as desired.***

	1	2	3	4	5	6	7	8	9	10	11	12
A	5 µM	5 µM	Sample 1	Sample 1	Sample 9	Sample 9	Sample 17	Sample 17	Sample 25	Sample 25	Sample 33	Sample 33
B	2.5 µM	2.5 µM	Sample 2	Sample 2	Sample 10	Sample 10	Sample 18	Sample 18	Sample 26	Sample 26	Sample 34	Sample 34
C	1.25 µM	1.25 µM	Sample 3	Sample 3	Sample 11	Sample 11	Sample 19	Sample 19	Sample 27	Sample 27	Sample 35	Sample 35
D	0.63 µM	0.63 µM	Sample 4	Sample 4	Sample 12	Sample 12	Sample 20	Sample 20	Sample 28	Sample 28	Sample 36	Sample 36
E	0.31 µM	0.31 µM	Sample 5	Sample 5	Sample 13	Sample 13	Sample 21	Sample 21	Sample 29	Sample 29	Sample 37	Sample 37
F	0.16 µM	0.16 µM	Sample 6	Sample 6	Sample 14	Sample 14	Sample 22	Sample 22	Sample 30	Sample 30	Sample 38	Sample 38
G	0.08 µM	0.08 µM	Sample 7	Sample 7	Sample 15	Sample 15	Sample 23	Sample 23	Sample 31	Sample 31	Sample 39	Sample 39
H	0 µM	0 µM	Sample 8	Sample 8	Sample 16	Sample 16	Sample 24	Sample 24	Sample 32	Sample 32	Sample 40	Sample 40

11. Incubate plate at room temperature for one hour.

12. Wash plate with PBS four times (4X) and on the final wash incubate the plate for 2 minutes before removing the wash solution. Ensure all wash buffer is removed before progressing to the next step.

13. Dilute HRP-goat anti-mouse in block solution by adding 12mL block solution to the HRP-goat anti-mouse bottle (HRP-goat anti-mouse bottle contains 12µL of 1000X HRP-goat anti-mouse) and mix well.

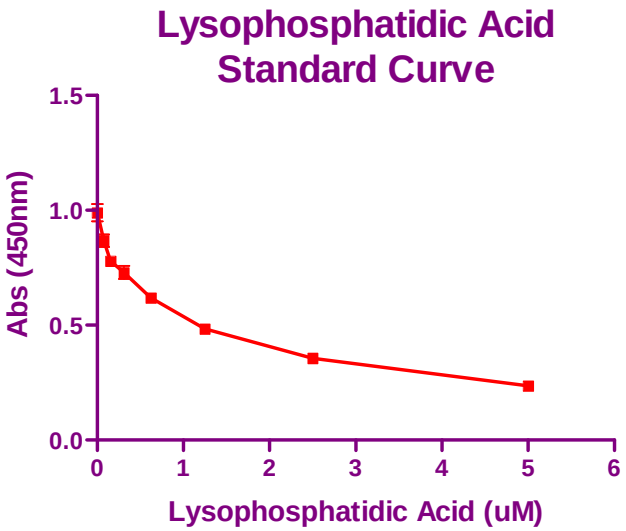
14. Add 100µL of diluted HRP-goat anti-mouse to each well of the microtiter plate (Nunc, Maxisorp) and incubate at room temperature for one hour. (place fresh microtiter plate seal on plate)

15. Wash plate with PBS four times (4X) and on the final wash incubate the plate for 2 minutes before removing the wash solution. Ensure all wash buffer is removed before progressing to the next step.

16. Add 100µL TMB substrate to each well of the microtiter plate (Nunc Maxisorp) and incubate for 4 to 8 minutes at room temperature.

17. Add 50µL H_2SO_4 to each well of the microtiter plate to stop the reaction.

18. Read plate at 450 nm.



*Figur*e. LPA competitive ELISA standard curve was graphed using point to point analysis. As little as 0.16µM LPA can be detected in 100µL of sample.

Assay Notes:

1. Ensure all samples are free from debris before adding to the plate.
2. Use care when washing microtiter plate. Inconsistent microtiter plate washing will result in higher assay variation.
3. All samples and standards must be diluted in delipidized human sera before use in the assay.
4. Be cautious of edge effects. Wells at the edge of the microtiter plate may exhibit increased variation from wells on the interior of the plate. If concerned about edge effect do not use wells on the exterior of the microtiter plate.
5. Diluted antibody and goat anti-mouse HRP are stable for 7 days at 4 ºC.
6. Ensure bottom of plate is clean before reading absorbance (wipe off smudges and debris).
7. Use caution when using delipidized human sera. Treat delipidized human sera as a potential biohazard and dispose of accordingly.

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