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ab273332 Lysophosphatidylcholine Assay Kit (Colorimetric/Fluorometric)

View Kit datasheet: https://www.abcam.com/ab273332
(use https://www.abcam.cn/ab273332 for china, or
https://www.abcam.co.jp/ab273332 for Japan)

For the determination of Lysophosphatidylcholine in adherent/suspension cells, tissue and other biological fluids.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Lysophosphatidylcholine Assay Kit (Colorimetric/Fluorometric) (ab273332) utilizes LPC-specific enzymes to generate an intermediate that then reacts with a probe, yielding a signal that can be quantified either colorimetrically or fluorometrically, and is proportional to the amount of LPC present in the sample.

When used as described, the assay is capable of detecting as little as 10 pmole of Lysophosphatidylcholine.

2. Protocol Summary

Prepare all reagents, samples and positive controls.

Prepare standards.

Add all samples to the appropriate wells.

Add Reaction Mix and Background Control Mix to the appropriate wells.

Incubate plate for 30 mins at 37°C in the dark.

Measure absorbance/fluorescence.

Determine Lysophosphatidylcholine using equation.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)
Lysis Buffer II	2 X 1 vial	-20°C
Assay Buffer 61	25 ml	-20°C
LPC Developer Mix	1 vial	-20°C
LPC Enzyme Mix	1 vial	-20°C
OxiRed™ Probe	0.2 mL	-20°C
LPC Standard	1 vial	-20°C

PLEASE NOTE: Assay Buffer 61 was previously labelled as Assay Buffer LXI and LPC Assay Buffer, and OxiRed™ Probe as OxiRed Probe and LPC Probe (in DMSO). The composition has not changed.

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Clear (colorimetric) or Black (fluorometric only) 96-well plate with flat bottom
- Multi-well Spectrophotometer
- Dounce Tissue Homogenizer
- 100% Methanol and Chloroform to make Upper Wash Layer for washing lipid extract

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

– Briefly centrifuge small vials at low speed prior to opening.

9.1 **Assay Buffer 61:**

Ready to use as supplied. Bring to room temperature before use. Store at +4°C protected from light.

9.2 **OxiRed™ Probe:**

Provided as a solution in DMSO. Store at -20°C, protected from light. Prior to use, warm solution to RT. After use, promptly retighten cap to minimize adsorption of airborne moisture.

9.3 **LPC Enzyme Mix:**

Reconstitute the vial with 220 µl Assay Buffer 61. Aliquot and store at -20°C. Keep on ice while in use and use reconstituted aliquots within two months.

9.4 **LPC Developer Mix:**

Reconstitute the vial with 220 µl Assay Buffer 61. Aliquot and store at -20°C. Keep on ice while in use and use reconstituted aliquots within two months.

9.5 **LPC Standard:**

Reconstitute with 200 µl Assay Buffer and mix thoroughly to generate a 2.5 mM LPC Standard solution. Aliquot and store at -20°C. Use within two months.

9.6 **Lysis Buffer II:**

Dilute 2 fold with dH_2O to generate 1X Lysis Buffer II for the assay.

9.7 **Upper Wash Layer (for washing lipid extract):**

Combine 5 ml methanol with 5 ml chloroform and 4.5 ml dH_2O, shake vigorously and allow mixture to separate into a lower layer and an Upper Wash Layer.

10. Sample Preparation

Serum and plasma preparation:

10.1 To 50 µl Sample, add 500 µl 100% methanol. Vortex for 1 min.

10.2 Place the Sample on ice for 10 mins and then centrifuge for 5 mins 10,000 *x g*, RT in a microcentrifuge. A pellet will be visible.

10.3 Collect the supernatant and transfer to a new tube without disturbing this pellet.

10.4 Evaporate the organic solvent at ≥37°C in a vacuum oven (or dry heat block within a fume hood) until the solvent evaporates completely.

10.5 Once fully dried, the extracted lipids will form a thin translucent film stuck to the walls of the tube.

10.6 Resuspend the dried lipid film in 50 µl of 1X Lysis Buffer II and vigorously vortex or sonicate to ensure solubilization.

10.7 If not being used immediately, resolubilized extracted lipids may be stored at -80°C for up to 1 week.

Tissue and cell pellets:

10.8 Thoroughly homogenize soft tissues (~10 mg wet tissue) or cultured cells (~2 x 10^6 cells) in 200 µl ice cold Assay Buffer 61 using a mechanical (Dounce) or ultrasonic probe homogenizer.

10.9 Perform sample lipid extraction according to the following protocol: Add 200 µl of the sample homogenate to a 15 ml conical polypropylene centrifuge tube, mix with 750 µl of a 1:2 mixture of chlorofom:methanol. Vortex thoroughly for 1 min. Add 250 µl chloroform to the sample/chloroform/methanol mix, vortex for 30 secs. Add 250 µl dH_2O to the tube, and again vortex to thoroughly mix and centrifuge sample at 1,500 *x g* for 10 mins at RT (25°C). Once the Sample/chloroform/methanol centrifugation has finished, three distinct layers will be visible: an upper phase containing methanol and aqueous fractions, a thin layer of precipitated protein, and the solubilized lipids in a lower organic phase. Aspirate and discard the upper phase, being careful not to remove the lower phase. Add 500 µl of the upper wash layer (prepared in Section 9) to the sample.

Mix vigorously and spin down at 1,500 *x g* for 10 mins at RT. Remove upper phase. This wash step may be repeated to further enrich lipids. After final wash(es), collect the lower phase through the protein layer with a pipette and transfer to a fresh tube.

10.10 Evaporate the organic solvent at ≥37°C in a vacuum oven (or dry heat block within a fume hood) until the solvent evaporates completely.

10.11 Once fully dried, the extracted lipids will form a thin translucent film stuck to the walls of the tube.

10.12 Resuspend the dried lipid film in 50-200 µl of 1X Lysis Buffer II and vigorously vortex or sonicate to ensure solubilization.

10.13 If not being used immediately, resolubilized extracted lipids may be stored at -80°C for up to 1 week.

Once samples are ready: Prepare duplicate wells, one Sample and one Background, by adding same volume (2-20 µl) of the lipid extract to the duplicate wells of a black 96-well plate (fluorometric) and 20-50 µl in clear 96-well plate (colorimetric detection) and adjust the volume in each well to 50 µl with Assay Buffer 61.

Δ Note: Once extracted, the lipid extracts can be stored at -80°C for future experiments

11. Standard Curve

For Colorimetric Assay:

11.1 Generate a 500 µM LPC Standard solution by diluting 50 µl of the LPC Standard (2.5 mM) with 200 µl of Assay Buffer 61.

11.2 Add 0, 2, 4, 6, 8 and 10 µl of the 500 µM LPC Standard solution into a series of wells in a clear 96-well plate to generate 0, 1, 2, 3, 4 and 5 nmol LPC Standard.

11.3 Bring the total volume of each well to 50 µl with Assay Buffer 61 to generate 0, 1, 2, 3, 4, and 5 nmol LPC per well.

Standard #	2.5 mM LPC Standard (µL)	Assay Buffer 61 (µL)	LPC (nmol/well)
1	0	50	0
2	2	48	1
3	4	46	2
4	6	44	3
5	8	42	4
6	10	40	5

For Fluorometric Assay:

11.4 Generate a 500 µM LPC Standard solution by diluting 50 µl of the LPC Standard (2.5 mM) with 200 µl of Assay Buffer 61.

11.5 Further dilute the 500 µM LPC solution by adding 50 µl to 450 µl Assay Buffer 61, yielding a 50 µM LPC Standard working solution.

11.6 Add 0, 2, 4, 6, 8, and 10 µl of the 50 µM LPC Standard to a series of wells in an opaque 96-well plate.
Bring the total volume of each well to 50 µl with Assay Buffer 61 to generate 0, 100, 200, 300, 400, and 500 pmol LPC per well.

Standard #	50 µM LPC Standard (µL)	Assay Buffer 61 (µL)	LPC (pmol/well)
1	0	50	0
2	2	48	100
3	4	46	200
4	6	44	300

5	8	42	400
6	10	40	500

12. Assay Procedure

12.1 **Reaction Mix:**
Mix enough reagents for the number of assays to be performed, including LPC Standard curve wells. For each test sample well, prepare 50 µl Reaction Mix and Sample Background Mix containing:

	Reaction mix (µl)	Background Control mix (µl)
Assay Buffer 61	44	46
LPC Enzyme Mix	2	---
LPC Developer Mix	2	2
OxiRed™ Probe*	2	2

* For fluorometric standards/application, reduce the amount of probe in the reaction mix to 0.4 µl, and adjust the buffer volume to 45.6 µl for Reaction/Standard Mix, 47.6 µl for Sample Background Mix.

12.2 Mix and add 50 µl of the Reaction Mix to each well containing standards and test samples.

12.3 For Sample Background wells, mix and add 50 µl of the Sample Background Mix to each well.

12.4 Incubate the plate for 30 mins at 37°C, protected from light and read the absorbance (570 nm) or fluorescence (Ex/Em = 535/587 nm) of all reactions, sample background and standard curve wells in endpoint mode.

13. Calculations

13.1 Subtract the 0 LPC Standard reading from all Standard curve readings, plot the Background-subtracted LPC Standard Curve and calculate the slope.

13.2 If Sample Background Control reading is significant, subtract the Background Control reading from its paired Sample reading.

13.3 Calculate the corrected absorbance/fluorescence of the Test Samples:

ΔOD/RFU = OD/RFUSample – OD/RFUBackground.

13.4 Apply the corrected ΔOD (or ΔRFU) value to the LPC Standard Curve to get B nmole (colorimetric) or pmol (fluorometric) LPC in the well.

$$[LPC] = \left(\frac{B}{V}\right) X\ D = nmol\ or\ pmol/\mu l$$

B = LPC amount from the Standard Curve (nmol or pmol)
V = Sample volume added into the reaction well (µl)
D = Dilution factor (D=1 for undiluted samples)

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

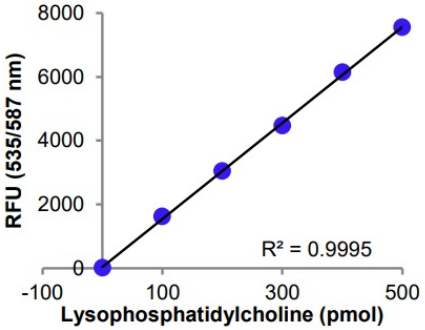

Figure 1. LPC Standard curve, fluorometric.

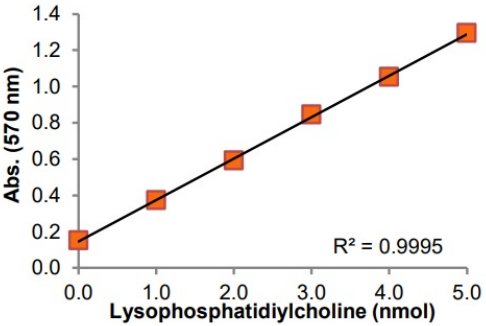

Figure 2. LPC Standard curve, colorimetric.

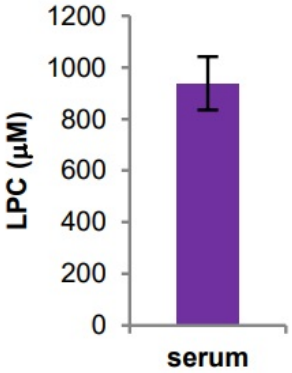

Figure 3. Determination of total LPC concentration in pooled human serum. Sample was analyzed according to the kit protocol. Values were determined with fluorometric measurements and are mean ± standard deviation of at least three independent determinations.

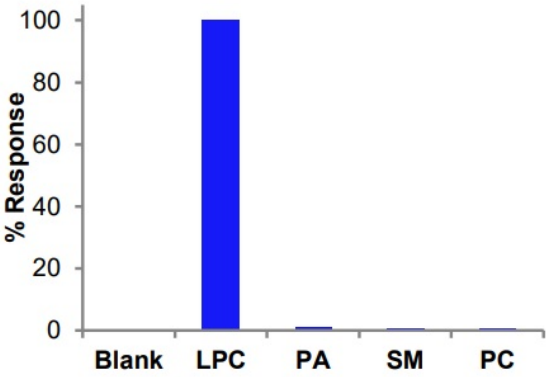

Figure 3. Specificity of the assay: PC = phosphatidylcholine, PA = phosphatidic acid, SM = sphingomyelin, LPC = lysophosphatidylcholine. 1 nmole of each was tested in the fluorometric assay

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)