Technical Bulletin

Lysophosphatidylcholine Assay Kit (Colorimetric/Fluorometric)

Catalog Number MAK413

Product Description

Lysophosphatidylcholine (LPC), also referred to as lysolecithin, is a phospholipid intermediate for which concentrations have been correlated with diseases such as cancer, atherosclerosis, and diabetes. Generally produced through the action of phospholipases on phosphatidylcholine, LPC consists of a glycerol backbone with a phosphocholine at one hydroxyl group and a single acyl chain attached to either the 1- or 2-position hydroxyl of the glycerol moiety. LPC is typically at concentrations in the high micromolar range in human serum and plasma and can activate second messengers such as Ca^{2+} and cyclic AMP. It is through these signaling pathways that LPC has been found to affect biological events such as the pro-inflammatory response and intestinal uptake.

The Lysophosphatidylcholine Assay Kit utilizes LPC-specific enzymes to generate an intermediate that 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. The method can detect as little as 10 pmole of lysophosphatidylcholine.

The kit is suitable for the measurement of lysophosphatidylcholine in tissue and cell lysates and biological fluids such as serum and plasma.

Components

The kit is sufficient for 100 colorimetric or fluorometric assays in 96-well plates.

- LPC Assay Buffer 25 mL
  Catalog Number MAK413A
- LPC Enzyme Mix 1 vial
  Catalog Number MAK413B
- LPC Developer 1 vial
  Catalog Number MAK413C
- LPC Probe (in DMSO) 200 µL
  Catalog Number MAK413D
- LPC Standard (0.5 µmol) 1 vial
  Catalog Number MAK413E
- 2x Lipid Resuspension Buffer 2 x 1 mL
  Catalog Number MAK413F

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Fluorescence multiwell plate reader
- Clear (colorimetric) or black (fluorometric) flat-bottom 96-well plates Cell culture or tissue culture treated plates are **not** recommended.
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Microcentrifuge capable of RCF ≥10,000 × *g*
- Methanol (MeOH) (Catalog Number M1775 or equivalent)
- Chloroform (Catalog Number C2432 or equivalent)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C, protected from light.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

LPC Assay Buffer: Allow to warm to room temperature prior to use. Chill an appropriate amount of LPC Assay Buffer for use in Sample Preparation. Store at 2-8 °C, protected from light.

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

LPC Enzyme Mix and LPC Developer: Reconstitute each vial with 220 µL of LPC Assay Buffer. Aliquot and store at -20 °C. Keep on ice while in use and use reconstituted aliquots within two months.

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

2x Lipid Resuspension Buffer: Dilute 2-fold with purified water to generate 1× Lipid Resuspension buffer for the assay.

Upper Wash Layer (for washing lipid extract): Combine 5 mL of methanol with 5 mL of chloroform (solvents not included) and 4.5 mL of purified water. Shake vigorously and allow mixture to separate into a lower layer and an upper layer. **Use the upper layer solvent as the Upper Wash Layer** in the Procedure.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

Serum and plasma

1. To 50 µL of serum or plasma sample, add 500 µL of 100% methanol (not included).
2. Vortex for 1 minute.
3. Place the sample on ice for 10 minutes.
4. Centrifuge for 5 minutes, 10,000 × *g*, at room temperature in a microcentrifuge. A pellet will be visible.
5. Collect the supernatant and transfer to a new tube without disturbing this pellet.
6. Evaporate the organic solvent at ≥37 °C in a vacuum oven (or dry heat block within a fume hood) until the solvent evaporates completely. Once fully dried, the extracted lipids will form a thin translucent film stuck to the walls of the tube.

7. Resuspend the dried lipid film in 50 µL of 1× Lipid Resuspension Buffer and vigorously vortex or sonicate to ensure solubilization.
8. If not being used immediately, resolubilized extracted lipids may be stored at -80 °C for up to one week.

Tissues and Cells

1. Thoroughly homogenize soft tissues (~10 mg wet tissue) or cultured cells (~2 × 10^6 cells) in 200 µL ice-cold LPC Assay Buffer using a mechanical (Dounce) or ultrasonic probe homogenizer.
2. Perform sample lipid extraction according to the following protocol:
   a. Add 200 µL of the sample homogenate to a 15 mL conical polypropylene centrifuge tube.
   b. Mix with 750 µL of a 1:2 mixture of chloroform: methanol (solvents not included).
   c. Vortex thoroughly for 1 minute.
   d. Add 250 µL of chloroform to the Sample/Chloroform/Methanol mixture from Step 2c.
   e. Vortex for 30 seconds.
   f. Add 250 µL of purified water to the mixture. Vortex to thoroughly mix.
   g. Centrifuge the sample at 1500 × *g* for 10 minutes at room temperature (25 °C).
3. After the sample lipid extraction and centrifugation, 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.
4. Aspirate and discard the upper phase, being careful not to remove the lower phase.
5. Add 500 µL of the **Upper Wash Layer** (prepared in the Reagent Preparation section) to the sample.
6. Mix vigorously and centrifuge at 1500 × *g* for 10 minutes at room temperature.
7. Aspirate and discard the upper phase, being careful not to remove the lower phase. This wash step may be repeated to further enrich lipids.
8. After final wash(es), collect the lower phase through the protein layer with a pipette and transfer to a fresh tube.
9. Evaporate the organic solvent at ≥37 °C in a vacuum oven (or dry heat block within a fume hood) until the solvent evaporates completely. Once fully dried, the extracted lipids will form a thin translucent film stuck to the walls of the tube.
10. Resuspend the dried lipid film in 50-200 µL of 1× Lipid Resuspension Buffer and vigorously vortex or sonicate to ensure solubilization.
11. If not being used immediately, resolubilized extracted lipids may be stored at -80 °C for up to one week.

For All Samples

Prepare duplicate wells, one Sample (S) and one Sample Background Control (SBC), by adding the same volume (2-20 µL) of the lipid extract to the duplicate wells of a black 96-well plate (for fluorometric detection) **or** the same volume (20-50 µL) to the duplicate wells of a clear 96-well plate (for colorimetric detection). Adjust the total volume in each well to 50 µL with LPC Assay Buffer. For unknown samples, test different amounts of sample to ensure the readings are within the Standard Curve range.

Colorimetric Standard Curve Preparation

Prepare a 500 µM LPC Standard by diluting 50 µL of the 2.5 mM LPC Standard with 200 µL of LPC Assay Buffer. Prepare LPC Standards for colorimetric assay according to Table 1. Mix well.

Table 1.
Preparation of LPC Standards for colorimetric assay

Well	500 µM LPC Standard	LPC Assay Buffer	LPC (nmol/well)
1	0 µL	50 µL	0
2	2 µL	48 µL	1
3	4 µL	46 µL	2
4	6 µL	44 µL	3
5	8 µL	42 µL	4
6	10 µL	40 µL	5

Fluorometric Standard Curve Preparation

1. Prepare a 500 µM LPC Standard by diluting 50 µL of the 2.5 mM LPC Standard with 200 µL of LPC Assay Buffer.
2. Further dilute the 500 µM LPC solution by adding 50 µL to 450 µL of LPC Assay Buffer, yielding a 50 µM LPC Standard working solution. Prepare LPC Standards for fluorometric assay according to Table 2. Mix well.

Table 2.
Preparation of LPC Standards for fluorometric assay

Well	50 µM LPC Standard	LPC Assay Buffer	LPC (pmol/well)
1	0 µL	50 µL	0
2	2 µL	48 µL	100
3	4 µL	46 µL	200
4	6 µL	44 µL	300
5	8 µL	42 µL	400
6	10 µL	40 µL	500

Reaction Mixes

1. Mix enough reagents for the number of assays to be performed.
   a. For each well containing Sample (S) or Standard, prepare 50 µL of appropriate Reaction Mix according to Table 3 (colorimetric) or Table 4 (fluorometric). Mix well.
   b. For each Sample Background Control (SBC) well, prepare 50 µL of appropriate Background Control Mix according to Table 3 (colorimetric) or Table 4 (fluorometric). Mix well.

Table 3.
Preparation of Reaction Mix for colorimetric assay

Reagent	Reaction Mix	Background Control Mix
LPC Assay Buffer	44 µL	46 µL
LPC Enzyme Mix	2 µL	-
LPC Developer Mix	2 µL	2 µL
LPC Probe	2 µL	2 µL

Table 4.
Preparation of Reaction Mix for fluorometric assay

Reagent	Reaction Mix	Background Control Mix
LPC Assay Buffer	45.6 µL	47.6 µL
LPC Enzyme Mix	2 µL	-
LPC Developer Mix	2 µL	2 µL
LPC Probe	0.4 µL	0.4 µL

2. Add 50 µL of the appropriate Reaction Mix to each well containing Standards and Sample (S). Mix well.
3. Add 50 µL of the Background Control Mix to each well containing Sample Background Control. Mix well.

Measurement

Incubate the plate for 30 minutes at 37 °C, protected from light. After incubation, read the absorbance at 570 nm (A_{570}) or fluorescence (RFU) at λ_{Ex} = 360 nm/ λ_{Em} = 440 nm for all Sample (S), Sample Background Control (SBC) and Standard wells in endpoint mode.

Results

1. Subtract the 0 LPC Standard reading from all Standard curve readings.
2. Plot the LPC Standard Curve and calculate the slope.
3. If Sample Background Control (SBC) reading is significant, subtract the Sample Background Control reading from its paired Sample reading. Calculate the corrected absorbance/fluorescence of each Sample (S):

   $\Delta A_{570} = A_{570\ Sample} - A_{570\ SBC}$

   $\Delta RFU = RFU_{\ Sample} - RFU_{\ SBC}$

4. Apply the corrected ΔA_{570} or ΔRFU value to the LPC Standard Curve to determine B nmole (colorimetric) or B pmol (fluorometric) LPC in the well.

Sample LPC Concentration [nmol/µL or mM (colorimetric) or pmol/µL or µM (fluorometric)] =

$(B/V) \times D$

where:

B = LPC amount from Standard Curve (in nmol or pmol)

V = Volume of sample added in the assay well (in µL)

D = Sample dilution factor (if applicable; D = 1 for undiluted samples)

Figure 1.
Typical LPC fluorometric Standard Curve

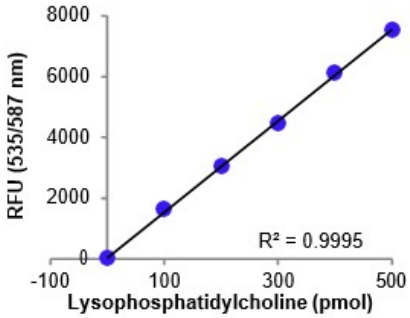


Figure 2.
Typical LPC colorimetric Standard Curve

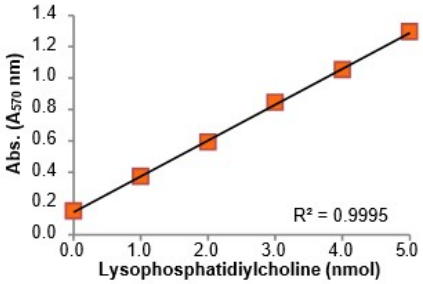


Figure 3.
Determination of total LPC concentration in pooled human serum. Sample was analyzed according to the kit protocol. Values were determined with fluorometric measurements and are mean ± standard deviation of at least three independent determinations.

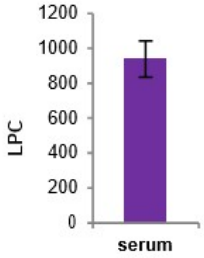
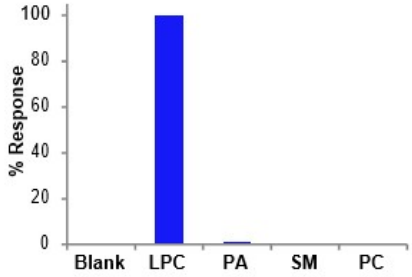


Figure 4.
Specificity of the assay:
PC = phosphatidylcholine,
PA = phosphatidic acid, SM = sphingomyelin,
LPC = lysophosphatidylcholine. 1 nmole of each was tested in the fluorometric assay following the kit protocol.



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