Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Lysophosphatidylcholine Assay Kit (Colorimetric/Fluorometric)

Rev 07/19

(Catalog # K735-100; 100 assays; Store at -20°C)

I. Introduction:

Lysophosphatidylcholine (LPC), also referred to as lysolecithin, is a phospholipid intermediate whose concentrations have been correlated with such diseases as cancer, artherosclerosis 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 is capable of activating second messengers such as Ca^{2+} and cyclic AMP. It is through these pathways that LPC has been found to affect such biological events as the pro-inflammatory response and intestinal uptake. BioVision's Lysophosphatidylcholine Assay Kit 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.

LPC $\xrightarrow{\text{Enzyme Mix}}$ Intermediate $\xrightarrow{\text{Developer/Probe}}$ Colorimetric (570 nm)/**Fluorescence ($\lambda_{ex/em}$ = 535 nm/587 nm)**

II. Applications:

- Measurement of LPC content of various tissue/cell extracts
- Determination of LPC concentration in biological fluids

III. Sample Type:

- Tissue and cell lysates
- Biological fluids (*e.g.* serum, plasma)

IV. Kit Contents:

Components	K735-100	Cap Code	Part Number
LPC Assay Buffer	25 ml	WM	K735-100-1
LPC Enzyme Mix	1 vial	Purple	K735-100-2
LPC Developer	1 vial	Green	K735-100-3
LPC Probe (in DMSO)	200 µl	Red	K735-100-4
LPC Standard (0.5 µmol)	1 vial	Yellow	K735-100-5
2X Lipid Resuspension Buffer	2 x 1 ml	Amber	K735-100-6

V. User Supplied Reagents and Equipment:

- Clear (colorimetric) or Black (fluorometric only) 96-well plate with flat bottom
- Multi-well Spectrophotometer
- Dounce Tissue Homogenizer (BV Cat. # 1998 or similar)
- 100% Methanol & Chloroform

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **LPC Assay Buffer:** Allow to warm to room temperature (RT) prior to use. Store at 4°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 RT. After use, promptly retighten cap to minimize adsorption of airborne moisture.
- **LPC Enzyme Mix and LPC Developer:** Reconstitute each vial with 220 µl 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 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.
- **2X Lipid Resuspension Buffer:** Dilute 2 fold with dH_2O to generate 1X Lipid Resuspension buffer for the assay.
- **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**.

VII. LPC Assay Protocol:

a. Sample Preparation:

Serum and plasma: To 50 µl Sample, add 500 µl 100% methanol. Vortex for 1 min. Place the Sample on ice for 10 min., and then centrifuge for 5 min, 10,000 x *g*, RT in a microcentrifuge. A pellet will be visible. C*ollect the supernatant* and transfer to a new tube without disturbing this pellet. 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. Resuspend the dried lipid film in 50 µl of 1X Lipid Resuspension Buffer and vigorously vortex or sonicate to ensure solubilization. *If not being used immediately, resolubilized extracted lipids may be stored at -80°C for up to 1 week*.

Tissues and cell pellets: Thoroughly homogenize soft tissues (~10 mg wet tissue) or cultured cells (~2 x 10^6 cells) in 200 µl ice cold LPC Assay Buffer using a mechanical (Dounce) or ultrasonic probe homogenizer (BV Cat. #1998 or similar). 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 sec. Add 250 µl dH_2O to the tube, and again vortex to thoroughly mix and centrifuge sample at 1500 x *g* for 10 min 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 (Section VI)** to the sample. Mix vigorously and spin down at 1500 x *g* for 10 minutes 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. 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. Re suspend the dried lipid film in 50-200 µl of 1X Lipid Resuspension Buffer (provided) and vigorously vortex or sonicate to ensure solubilization. *If not being used immediately, resolubilized extracted lipids may be stored at -80°C for up to 1 week.*

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 LPC Assay Buffer.

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

2. **Standard Curve Preparation:** Generate a 500 µM LPC Standard solution by diluting 50 µl of the LPC Standard (2.5 mM) with 200 µl of LPC Assay Buffer. **Colorimetric assay**: 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 & 5 nmol LPC Standard. **Fluorometric assay:** Further dilute the 500 µM LPC solution by adding 50 µl to 450 µl LPC Assay Buffer, yielding a 50 µM LPC Standard working solution. 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 LPC Assay Buffer to generate 0, 100, 200, 300, 400, and 500 pmol LPC per well.
3. **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/Standard Mix	Sample Background 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

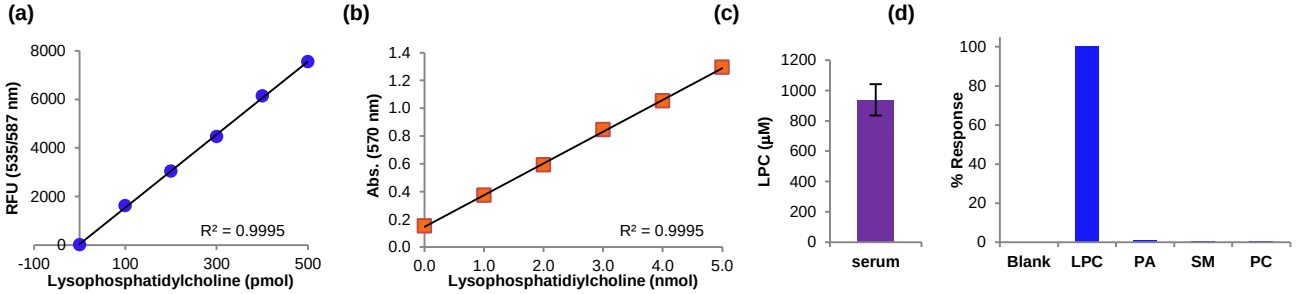
Note:** For ***fluorometric standards/application, reduce the amount of probe in the reaction mix to 0.4 µl, and adjust the buffer volume to 45.6 µl for Reaction/Standard Mix, 47.6 µl for Sample Background Mix.

Mix and add 50 µl of the Reaction Mix to each well containing standards and test samples. For Sample Background wells, mix and add 50 µl of the Sample Background Mix to each well.

4. **Measurement:** Incubate the plate for 30 min 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.
5. **Calculation:** Subtract the 0 LPC Standard reading from all Standard curve readings, plot the Background-subtracted LPC Standard Curve and calculate the slope. If Sample Background Control reading is significant, subtract the Background Control reading from its paired Sample reading. Calculate the corrected absorbance/fluorescence of the Test Samples $\Delta OD/RFU = OD/RFU_{Sample} - OD/RFU_{Background}$. Apply the corrected ΔOD (or ΔRFU) value to the LPC Standard Curve to get *B* nmole (colorimetric) or pmol (fluorometric) LPC in the well.

Sample LPC Concentration = (*B* / *V*) x *D* = (nmol or pmol)/µl ≡ mM or µM

Where: ***B*** = LPC amount from Standard Curve (in nmol/pmol)
V = Sample volume added into the reaction well (in µl)
D = Sample dilution factor (if applicable)



Figures: (a) LPC Standard curve, fluorometric and **(b)** colorimetric. **(c)** 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. **(d)** Specificity of the assay: PC = phosphatidylcholine, PA = phosphatidic acid, SM = sphingomyelin, LPC = lysophosphatidylcholine. 1 nmole of each was tested in the fluorometric assay.

VIII. RELATED PRODUCTS:

Phospholipase A2 Activity Assay Kit (Fluorometric) (K400)
Phospholipase D Activity Colorimetric Assay Kit (K725)
Cardiolipin Assay Kit (Fluorometric) (K944)
Phosphatidic Acid Assay Kit (K748)
Acid Sphingomyelinase Activity Colorimetric Assay Kit (K598)
Cholinesterase Activity Assay Kit (Colorimetric) (K975)
Acetylcholinesterase Activity Colorimetric Assay Kit (K764)
Phosphatidylcholine Colorimetric/Fluorometric Assay Kit (K576)
Phospholipid Assay Kit (Colorimetric/Fluorometric) (K351)
Glycerophosphorylcholine Assay Kit (C/F) (K433)
Alkaline Sphingomyelinase Activity Assay Kit (Colorimetric) (K987)

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