NanoPro™ Assay: Phospholipase C Gamma 1 (PLCγ1)

Cell Biosciences™

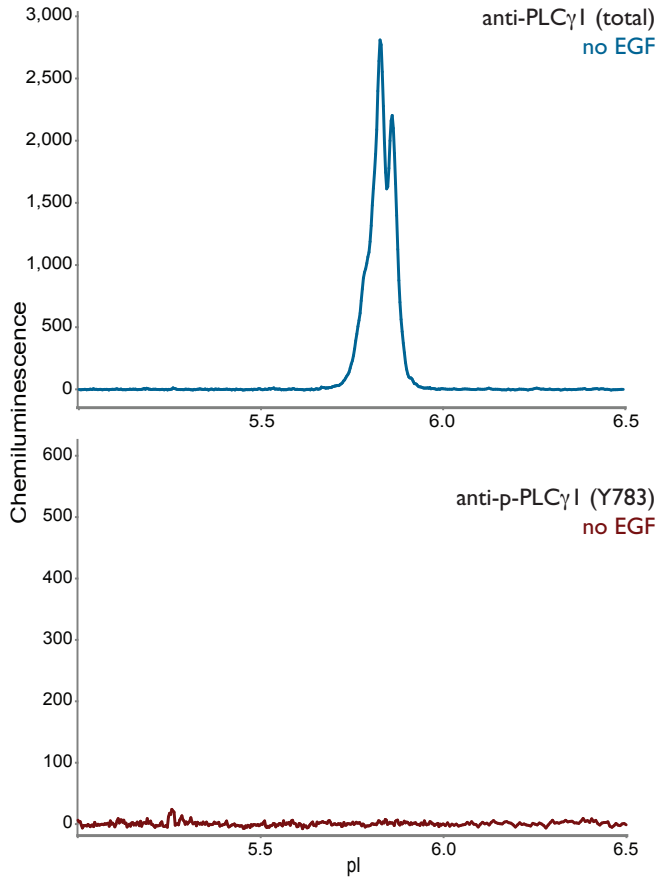
APPLICATION BRIEF No. 1014

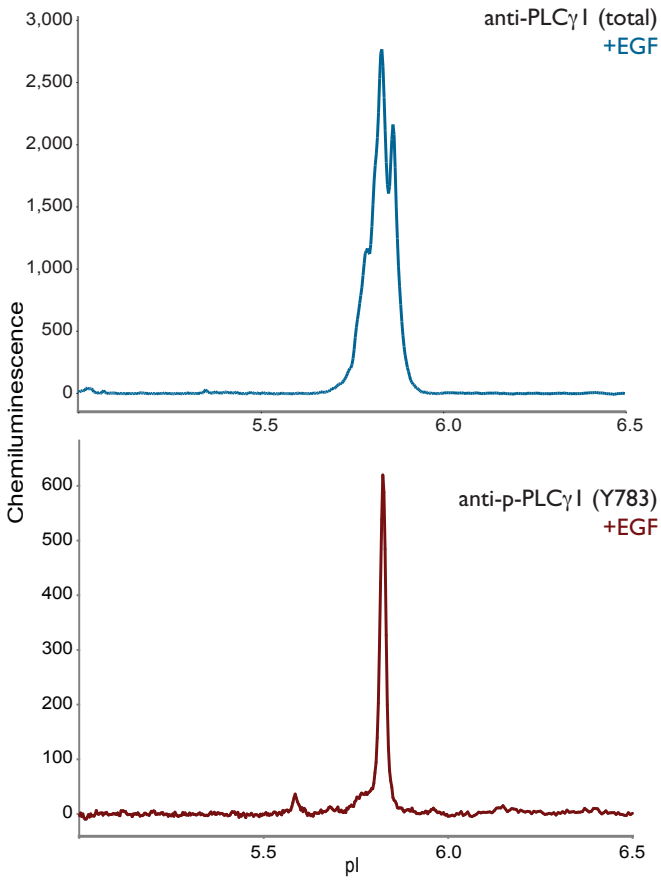
SUMMARY

Primary Antibody: Anti-PLCγ1 (Cell Signaling Technology, cat# 2822) and anti-phospho PLCγ1 (Abcam, cat# ab53125)
Detection Antibody: Anti-Rabbit HRP (Cell Biosciences, p/n 040-656)

Phospholipase C (PLC) catalyzes the hydrolysis of phosphatidylinositol 4, 5-bisphosphate (PIP2) to produce the metabolite second messenger molecules inositol 1, 4, 5-trisphosphate (IP3) and diacylglycerol (DAG). Increase of IP3 results in elevated intracellular free Ca^{2+}. PLC's are activated through G-protein coupled receptor stimulation as well as tyrosine receptor kinase activation and therefore bridge both important signaling pathways. The PLC family consists of 12 isoforms with different roles in signaling. For example, PLCγ1 forms a complex with activated EGF receptors, which leads to the phosphorylation of PLCγ at Tyr771, 783 and 1245. Here we detect the phosphorylation of PLCγ1 in HEK293 cells in response to EGF treatment.

RESULTS





EGF treatment results in increased PLCγ1 phosphorylation in HEK293 cells

HEK293 cells were treated -/+ 50 ng/mL EGF for 15 minutes. EGF treatment resulted in a dramatic increase in a pI 5.8 peak detected with the anti-phospho (Y783) PLCγ1 antibody (right panel, lower trace). Additionally, a slight shoulder in the total PLCγ1 peak profile near pI 5.8 was detected with the anti-PLCγ1 (total) antibody (right panel, upper trace). Similar peak profiles have been generated in EGF-treated HeLa cells and serum-treated U937 cells (data not shown).

NOTE: Detection of the chemiluminescent signal produced is relative. Absolute units may vary depending on cell line, treatment and assay conditions.

PROTOCOL

Cell Preparation

Cell culture: HEK293 cells (ATCC, cat# CRL-1573) were cultured in EMEM (ATCC, cat# 30-2003) containing 10% FBS (Irvine Scientific, cat# 3000-A) and 1x Penicillin/Streptomycin/Glutamine (JRS Scientific, cat# 20020). Cells were split 1:5 every 3 days using 0.25% Trypsin (Cellgro, cat# 25-053-CI) at 37 °C for 3–5 minutes. Data shown from cells at passage 5.

Pre-treatment: Before EGF stimulation, cells were placed at 37 °C, 5% CO_2 overnight in starvation medium containing MEM without serum.

Treatment: 50 ng/mL EGF in MEM without serum for 15 minutes at 37 °C, 5% CO_2.

Lysis buffer: Bicine/CHAPS Lysis Buffer (Cell Biosciences, p/n 040-764) plus 1x DMSO Inhibitor Mix (Cell Biosciences, p/n 040-510) and 1x Aqueous Inhibitor Mix (Cell Biosciences, p/n 040-482).

Lysis details: Wash cells with 10 mL of ice-cold PBS (Cellgro, cat# 21-031-CV), aspirate well. Add 400 µL ice-cold lysis buffer to 10-cm plate on ice, swirl around to ensure good coverage, and incubate 10 minutes on ice. Scrape plate, pipet up and down to mix. Transfer lysate to microfuge tube, lyse for an additional 30 minutes on ice. Clarify by centrifugation (14,000 x g, 15 minutes) in a cooled centrifuge. Transfer supernatant to a fresh microfuge tube. Immediately aliquot supernatant (10–30 µL) on ice and snap freeze on dry ice.

Storage: -80 °C

Assay Reagents

NOTE: For specifics on sample preparation, please consult the addendum to this document.

Protein concentration: 0.08 mg/mL final in capillary by BCA assay

Sample diluent: Sample Diluent (Cell Biosciences, p/n 040-649) plus 1x DMSO Inhibitor Mix

Ampholyte premix: Premix 5-8 (nested) (Cell Biosciences Premix G1, p/n 040-643 or Premix G2, p/n 040-972)

pI standards: pI Standard Ladder 3 (Cell Biosciences, p/n 040-646)

Wash: Wash Buffer (Cell Biosciences, p/n 040-654)

Primary antibody: Anti-PLCγ1 (Cell Signaling Technology, cat# 2822) and anti-phospho PLCγ1 (Abcam, cat# ab53125), both 1:50 in Antibody Diluent (Cell Biosciences, p/n 040-309)

Detection antibody: Anti-Rabbit HRP (Cell Biosciences, p/n 040-656), 1:100 in Antibody Diluent

Anolyte: Phosphoric Acid, 10 mM (Cell Biosciences, p/n 040-650)

Catholyte: Sodium Hydroxide, 100 mM (Cell Biosciences, p/n 040-651)

Luminol/Peroxide: Mixed 1:1 (Cell Biosciences, p/n 040-652 and p/n 040-653)

NOTE: NanoPro XDR Assays are now available with optional reagents to provide optimized assay sensitivity, extended dynamic range and both low and high end signal enrichment. Additionally, Premix G2 provides enhanced protein resolution. For more information, visit cellbiosciences.com, contact your Cell Biosciences' Field Applications Specialist or call Technical Support at (888) 607-9692.

Assay Conditions

System: NanoPro 1000

Sample loading time: 10 seconds (Premix G1), 25 seconds (Premix G2)

Focus conditions: 15000 µW, 40 minutes (Premix G1) or 21000 µW, 40 minutes (Premix G2)

Immobilization: 80 seconds

Wash 1: 2 x 150 seconds (default)

Primary antibody incubation: 120 minutes

Wash 2: 2 x 150 seconds (default)

Detection antibody incubation: 60 minutes

Wash 3: 2 x 150 seconds (default)

Chemiluminescence exposure: 60, 120, and 240 seconds

Our favorite antibodies

Anti-PLCγ1 (Cell Signaling Technology, cat# 2822)
Anti-phospho PLCγ1 (Abcam, cat# ab53125)

Other antibody suggestions

Anti-PLCγ1 (Abcam, cat# ab52200)
Anti-phospho PLCγ1 (Millipore, cat# 072134)

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SAMPLE PREPARATION ADDENDUM

Sample preparation procedures are different depending on the premix used. The following updated instructions provide guidance on sample preparation for both Premix G1 and Premix G2.

Step	Premix G1 Procedure	Premix G2 Procedure
Step 1	Dilute lysate with sample diluents to 0.1 mg/mL.	Dilute lysate with sample diluents to 0.2 mg/mL.
Step 2	In a separate tube, mix Premix G1and pI standards.	In a separate tube, mix Premix G2 and pI standards.
Step 3	Mix equal parts of diluted lysate prepared in Step 1 with the Premix G1 + pI Standards prepared in Step 2 (1:1 ratio) to create final protein concentration of 0.05 mg/mL.	Mix 1 part diluted lysate prepared in Step 1 with 3 parts Premix G2 + pI Standards prepared in Step 2 (1:3 ratio) to create final protein concentration of 0.05 mg/mL.

NOTES: When working with Premix G2, thorough mixing and vortexing during sample preparation is required.
Additional sample volume may be required, 12-20 uL per sample well is recommended.
Centrifugation of the sample plate (3000 x g, 10 minutes) is required.

For further assistance, please contact your Cell Biosciences' Field Applications Specialist or Technical Support at (888) 607-9692.

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