Cell Biosciences™

NanoPro™ Assay: Src

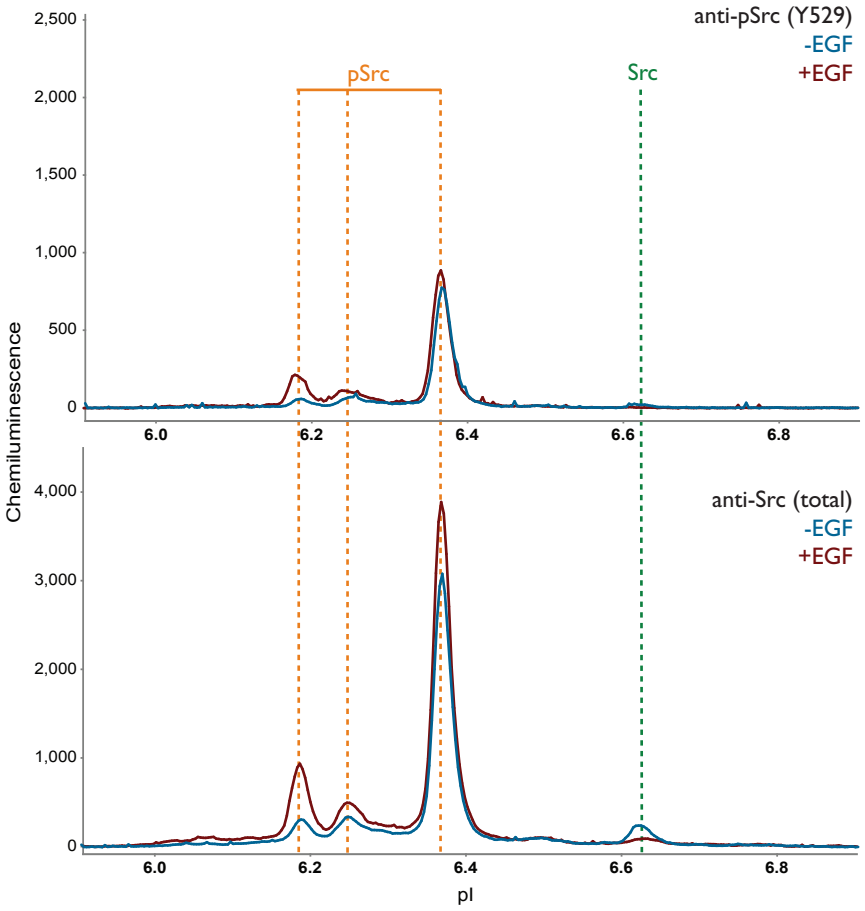
APPLICATION BRIEF No. 1018

SUMMARY

Primary Antibody: Anti-Src (Abcam, cat# ab47405), Anti-phospho Src (Tyr 529) (Cell Signaling Technology, cat# 2105)
Detection Antibody: Anti-Rabbit HRP (Cell Biosciences, p/n 040-656)

Src is involved in regulating growth and differentiation in eukaryotic cells. Src activity is regulated by tyrosine phosphorylation at two sites, but with opposing effects. Phosphorylation of Tyr416 in the activation loop of the kinase domain by Csk upregulates enzyme activity, whereas phosphorylation of Tyr529 in the carboxy-terminal tail renders the enzyme less active. We evaluated Src response to EGF in A431 cells.

RESULTS



EGF treatment of A431 cells results in a small, but reproducible, increase in Src phosphorylation

A431 cells were treated -/+ 100 ng/mL EGF for 10 minutes. The profiles generated with the Anti-phospho Src (Y529) Antibody (Cell Signaling Technology, cat# 2105) showed an increase in the acidic peaks between pI 6.2 and 6.4 (upper traces, labeled pSrc). The Anti-Src (total) Antibody (Abcam, cat# ab47405) also detected peaks between pI 6.2 and 6.4 that increased upon EGF treatment (lower traces, labeled pSrc). In addition, the anti-Src (total) antibody detected a peak at pI 6.6 which decreased after EGF treatment (lower traces, labeled Src). These antibodies have been used to generate similar profiles in HEK293 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: A431 cells (ATCC, cat# CCL-1555) were cultured in DMEM (Irvine Scientific, cat#30-2002) containing 10% FBS (Hyclone, cat# 1677-006) 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 to dislodge. 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 DMEM with no additives.

Treatment: 100 ng/mL EGF (Sigma, cat# E1257) in DMEM for 10 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: 0.06 mg/mL final in capillary by BCA assay

Sample diluent: Bicine/CHAPS Lysis Buffer plus 1x DMSO Inhibitor Mix

Ampholyte premix: Premix 5-8 (Cell Biosciences Premix G1, p/n 040-327 or Premix G2, p/n 040-973)

pI standards: pI Standard Ladder 3 (Cell Biosciences, p/n 040-646) plus pI Standard 5.5 (Cell Biosciences, p/n 040-028)

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

Primary antibody: Anti-Src (Abcam, cat# ab47405) and Anti-phospho Src (Tyr529, Cell Signaling Technology, cat# 2105), 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: 120 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-Src (Abcam, cat# ab47405)
Anti-phospho Src (Tyr529) (Cell Signaling Technology, cat# 2105)

Other antibody suggestions

Anti-Src (Assay Design, cat# 905-678)
Anti-Src (Cell Signaling Technology, cat# 2108)
Anti-Src (Cell Signaling Technology, cat# 2109)
Anti-phospho Src (Tyr529) (Abcam, cat# ab4817)
Anti-phospho Src (Tyr418) (Cell Signaling Technology, cat# 2101)
Anti-phospho Src (Abcam, cat# ab4816)

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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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