Cell Biosciences™

NanoPro™ Assay: p-p27/Kipl

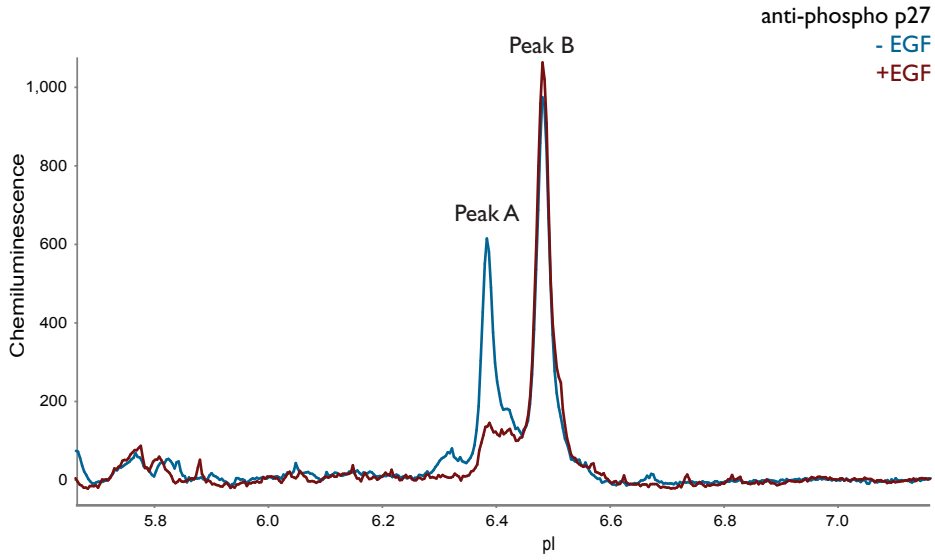
APPLICATION BRIEF No. 1020

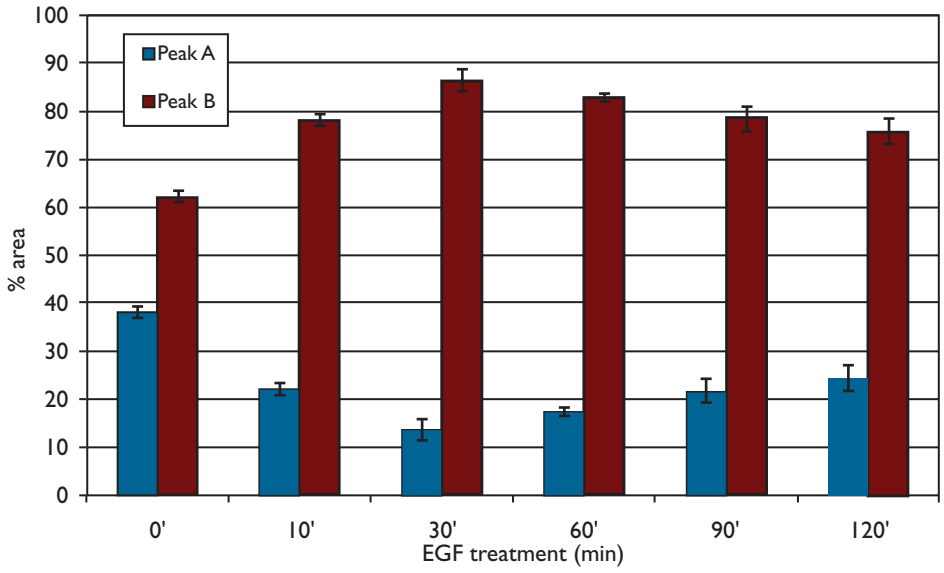
SUMMARY

Primary Antibody: Anti-phospho p27 (Abcam, cat# ab60019)
Detection Antibody: Anti-Rabbit HRP (Cell Biosciences, p/n 040-656)

p27, also known as Kip1, is a cell cycle regulatory/inhibitory protein. It is similar to other members of the Cip/Kip family which includes the p21Cip1/Waf1 and p57Kip2 genes. p27 shares its functional characteristic of being able to bind several different classes of Cyclin and CDK molecules, acting as a CDK inhibitor. We show the response of p27 to EGF treatment in MCF10A cells.

RESULTS





Specific p27 phosphorylation is reduced in a time-dependent manner upon EGF treatment in MCF10A cells

The figure at left shows representative profiles generated using an anti-phospho p27 antibody with untreated (blue trace) and maximally-stimulated (600 ng/mL EGF for 30 minutes, red trace) MCF10A cells.

The bar graphs quantify relative peak area changes for phospho peaks A (blue) and B (red) over time (n=4). Peaks A and B both collapse upon phosphatase treatment, confirming their phospho-peak identities (data not shown). In addition, a third peak around pI 6.8 appears after phosphatase treatment. This peak has been putatively identified as non-phospho p27 by detection with Anti-total p27 (Santa Cruz Biotechnology, cat# sc-528; 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: MCF10A cells (ATCC, cat# CRL-10317) were cultured in MEGM (Lonza, cat# CC3151) containing 10% FBS (Irvine Scientific, cat# 3000-A), 1x Penicillin/Streptomycin/Glutamine (JRS Scientific, cat# 20020), and MEGM SingleQuots (Lonza, cat# CC4136). 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 8.

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

Treatment: 600 ng/mL EGF in starvation medium for 30 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: 0.1 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-phospho p27 (Abcam, cat# ab60019), 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-p27 (total) (Santa Cruz Biotechnology, cat# sc-528)
Anti-p27 (total) (Abcam, cat# ab7961)
Anti-phospho p27 (Abcam, cat# ab60019 and cat# ab62364)

Other antibody suggestions

Anti-p27 (total) (Millipore, cat# 06-445)
Anti-p27 (total) (Rockland, cat# 100-401-172)

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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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