NanoPro™ Assay: p21 Protein-activated Kinase 2 (PAK 2)

Cell Biosciences™

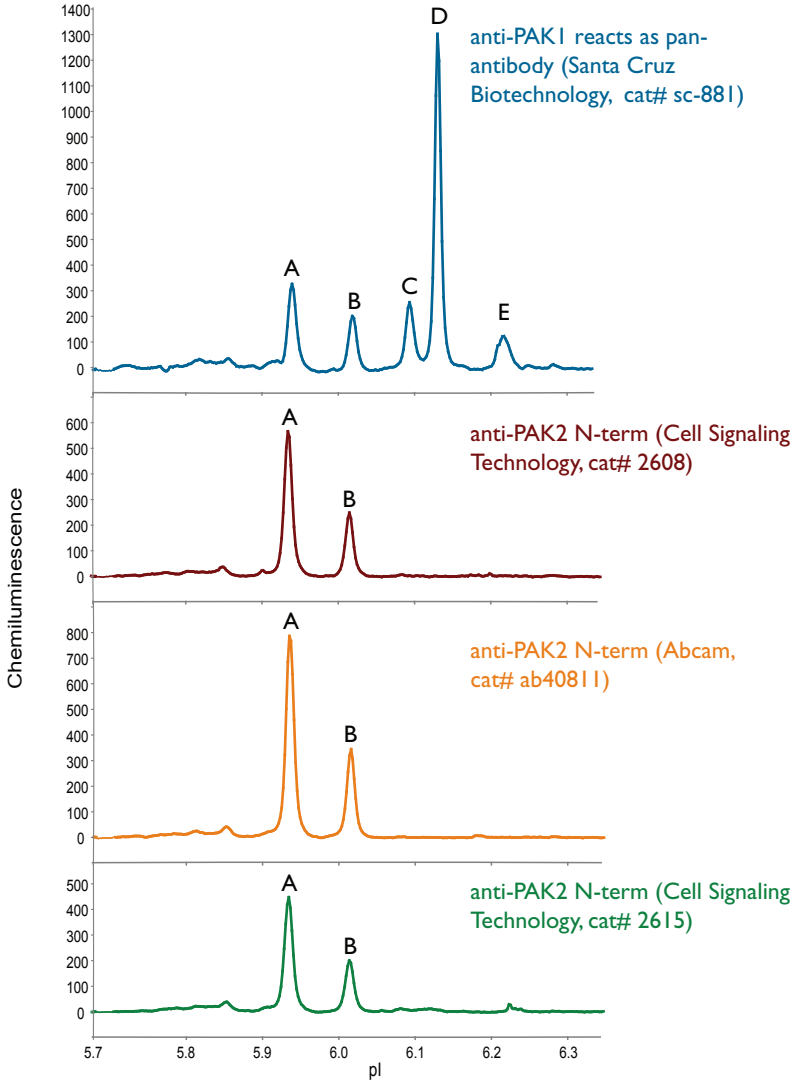
APPLICATION BRIEF No. 1003

SUMMARY

Primary Antibody: Anti-PAK1 reacts as pan-antibody (Santa Cruz Biotechnolgy, cat# sc-881) and
Anti-PAK2 (Cell Signaling Technology, cat# 2608 and cat# 2615, Abcam, cat# ab40811)
Detection Antibody: Anti-Rabbit HRP (Cell Biosciences, p/n 040-656)

The p21 activated kinase (PAK) proteins are a family of serine/threonine kinases that serve as targets for the small GTP binding proteins, CDC42 and RAC1, and have been implicated in a wide range of biological activities. PAK2 is a member of the PAK subfamily I including PAK1 (α-PAK), PAK2 (γ-PAK, PAKθ, hPAK65), and PAK3 (β-PAK). The kinase domains within a subfamily show a high degree of sequence identity, and all PAK proteins bind GTP-bound Rho family members at the amino-terminal p21-binding domain (PBD). Our data in HeLa cells show a pattern of 5 peaks using a pan-PAK1/2/3 antibody of which two are picked up consistently by 3 different specific PAK2 antibodies identifying them as PAK2 peaks.

RESULTS



4E-BP2 phosphorylation is induced by EGF stimulation in MCF10A cells

HeLa lysates probed with the pan-PAK antibody sc881 showed five putative PAK peaks labeled A–E. Peaks A and B were also recognized by three independent PAK2 specific antibodies shown, identifying peaks A and B as PAK2 peaks.

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: HeLa cells (ATCC, cat# CCL-2) were cultured in DMEM (ATCC, cat# 30-2002) 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 (Mediatech, 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 DMEM without serum.

Treatment: 50 ng/mL EGF (Millipore, cat# 01-107) in serum free DMEM for 5 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: 0.05 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 5-6 (Cell Biosciences Premix G1, p/n 040-763 or Premix G2, p/n 040-971)

pI standards: pI Standards 4.92, 5.5, 6.4 (Cell Biosciences, p/n 040-027, p/n 040-028, p/n 040-030), 1:100

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

Primary antibody: Anti-PAK1 (Santa Cruz Biotechnology, cat# sc-881, reacts with PAK1, 2, 3), Anti-PAK2 (Cell Signaling Technologies, cat# 2608 and cat# 2615 and Abcam, cat# ab40811), all 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: 60 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-PAK1 (reacts as pan PAK ab) (Santa Cruz Biotechnology, cat# sc-881)
Anti-PAK2 (Cell Signaling Technology, cat# 2608)
Anti-PAK2 (Cell Signaling Technology, cat# 2615)
Anti-PAK2 (Abcam, cat# ab40811)

Other antibody suggestions

Anti-PAK1 (Santa Cruz Biotechnology, cat# sc-11394)
Anti-PAK1 (Santa Cruz Biotechnology, cat# sc-882)
Anti-PAK1 (Epitomics, cat# EP656Y)
Anti-PAK3 (Cell Signaling Technology, cat# 2609)
Anti-Phospho-PAK1/2/3 (Millipore, cat# 09-258)
Anti-Phospho-PAK1/2/3 (Phospho Solutions, cat# p187-402)

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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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