Cell Biosciences™

NanoPro™ Assay: ERK1/2

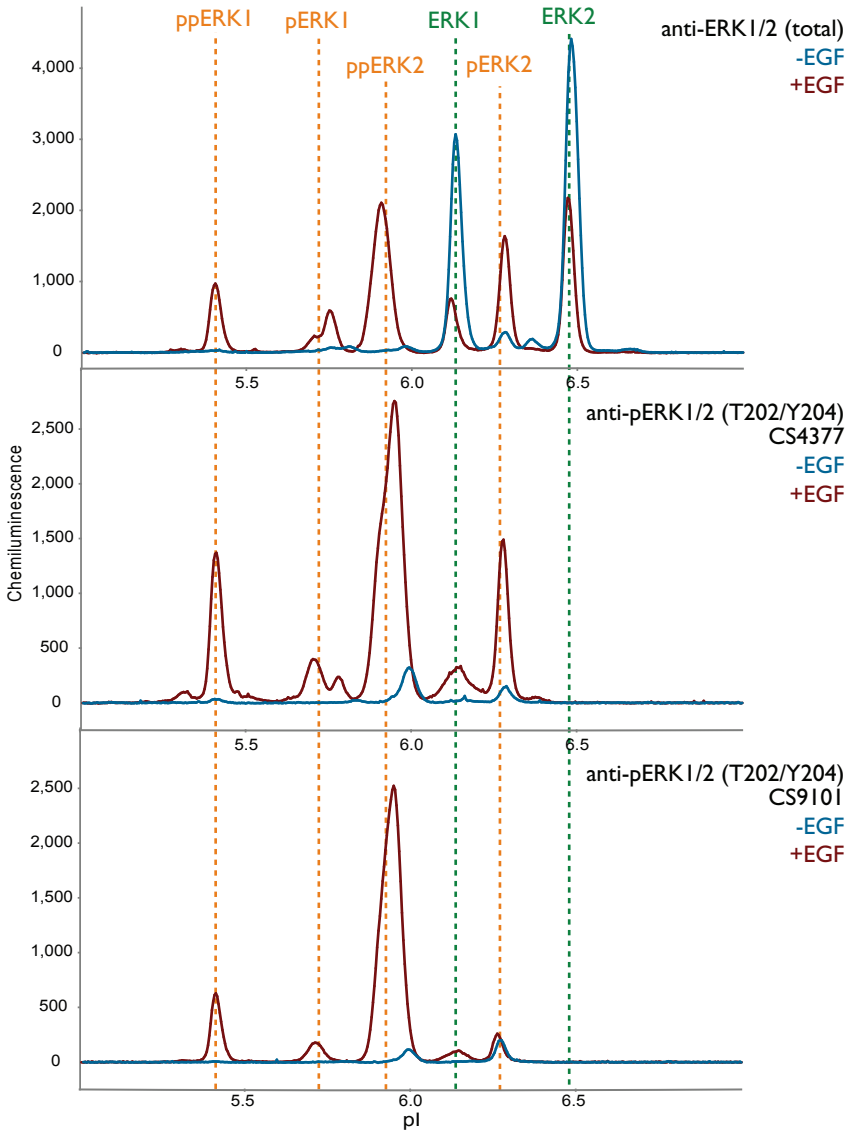
APPLICATION BRIEF No. 1015

SUMMARY

Primary Antibody: Anti-ERK1/2 (Millipore, cat# 06-182), Anti-phospho ERK (Cell Signaling Technology, cat# 9101) and
Anti-phospho ERK (Cell Signaling Technology, cat# 4377)
Detection Antibody: Anti-Rabbit HRP (Cell Biosciences, p/n 040-656)

Extracellular signal-regulated kinases (ERK) or classical MAP kinases are widely expressed intracellular signaling molecules involved in regulation of meiosis, mitosis, and post-mitotic functions in differentiated cells. Many different stimuli including growth factors, cytokines, virus infection, ligands for heterotrimeric G protein-coupled receptors, transforming agents, and carcinogens activate the ERK pathway. We show an exampleof ERK phosphorylation in MCF10A cells in response to treatment with epidermal growth factor (EGF).

RESULTS



EGF stimulation results in increased ERK1/2 phosphorylation in MCF10A cells

MCF10A cells were treated -/+ 600 ng/mL EGF for 10 minutes. Non-phosphorylated ERK1 and ERK2 were the dominant ERK species present in untreated MCF10A cells, as detected using the Millipore 06-182 anti-ERK1/2 (total) antibody (upper panel, blue trace). All three antibodies detected increased signal from phosphorylated ERK1 and phosphorylated ERK2 isoforms after EGF stimulation (red traces in upper, middle, and lower panels). However, the two anti-pERK1/2 antibodies shown in the middle and lower traces exhibited markedly different selectivities. Whereas the CS4377 recognized both the single- and dual-phosphorylated forms of ERK1/2, the CS9101 antibody preferentially recognized the dual-phosphorylated species. This type of detailed characterization of antibody selectivity is made possible by the IEF separation step in NanoPro assays.

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 5% FBS (Hyclone, cat# 1677-006), 1x Penicillin/Streptomycin/Glutamine (JRS Scientific, cat# 20020) and MEGM SingleQuot (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 5.

Pre-treatment: Before EGF stimulation, cells were placed at 37 °C, 5% CO_2 overnight in starvation medium containing MEGM minus FBS and growth supplements.

Treatment: 600 ng/mL EGF (Sigma, cat# E1257) in MEGM 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: 0.05 mg/mL final in capillary by BCA assay
Sample diluent: Bicine/CHAPS Lysis Buffer (Cell Biosciences, p/n 040-764) 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)
Wash: Wash Buffer (Cell Biosciences, p/n 040-654)
Primary antibody: Anti-ERK1/2 (Millipore, cat# 06-182), 1:200 and Anti-phospho ERK (Cell Signaling Technology, cat# 9101 or cat# 4377), 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-ERK1/2 (Millipore, cat# 06-182)
Anti-phospho ERK (Cell Signaling Technology, cat# 9101)
Anti-phospho ERK (Cell Signaling Technology, cat# 4377)

Other antibody suggestions

Anti-ERK1 (Millipore, cat# 05-754)
Anti-ERK2 (Biolegend, cat# 624202)
Anti-ERK2 (Millipore, cat# 06-333)

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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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