Cell Biosciences™

NanoPro™ Assay: MEK1

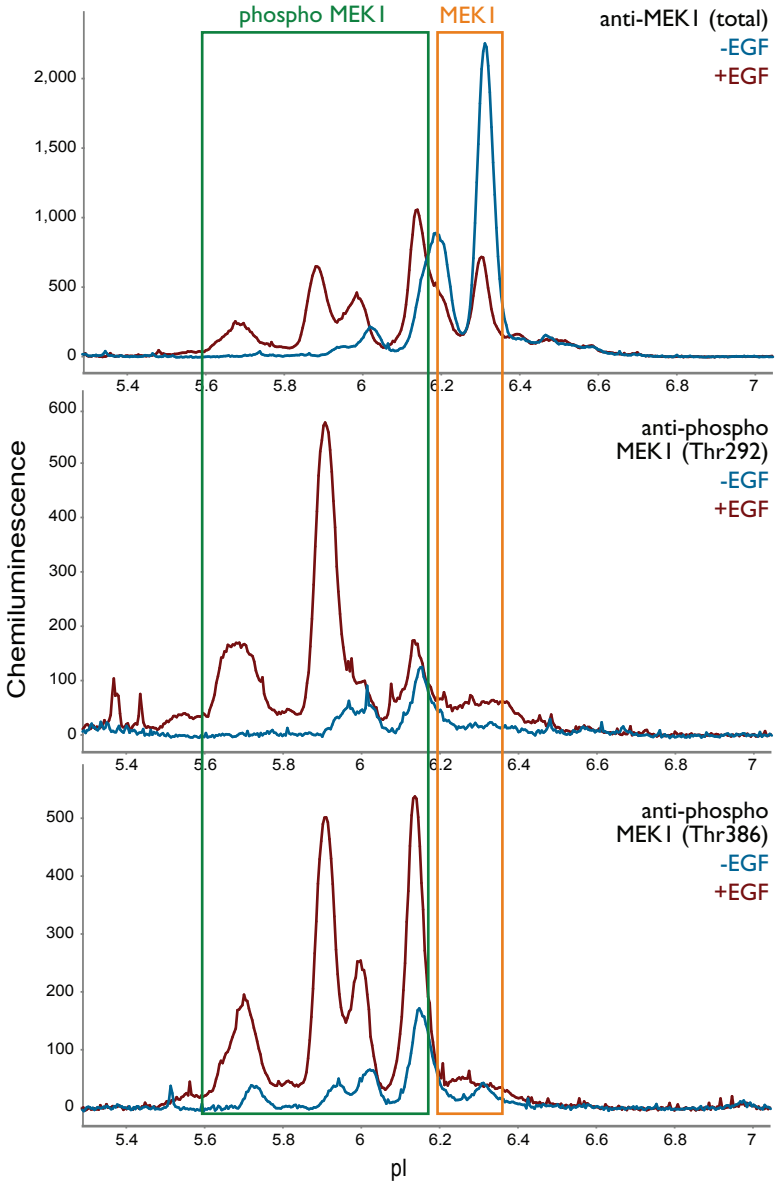
APPLICATION BRIEF No. 1016

SUMMARY

Primary Antibody: Anti-MEK1 (Millipore, cat# 07-641), Anti-phospho MEK1 (Thr292) (Millipore, cat# 07-852) and Anti-phospho MEK1 (Thr386) (Phospho Solution, cat# p180-386)
Detection Antibody: Anti-Rabbit HRP (Cell Biosciences, p/n 040-656)

Dual-specificity mitogen-activated protein kinases (MEK) are members of the dual-specificity protein kinase family, which act upstream from the classical MAP kinases through phosphorylation and thus activation of ERK1 and ERK2 in response to a wide variety of extra- and intracellular signals. While the functions of MEK1 and MEK2 are very similar, these kinases differ significantly in the way they are regulated. For example, serum addition can specifically induce MEK1 activity in CHO cells. By contrast, MEK2 appears to be the functionally predominant isoform in formyl-methionyl-leucyl-phenylalanine treated neutrophils. Here we show MEK1 activation in MCF10A cells treated with EGF.

RESULTS



EGF stimulation results in increased MEK1 phosphorylation in MCF10A cells

MCF10A cells were treated -/+ EGF (600 ng/mL, 10 minutes). Several phospho MEK1 peaks (green box) increased with EGF treatment, as detected by an anti-phospho MEK1 antibody (bottom two traces) and anti-total MEK1 antibody (top trace). The non-phospho peaks at pI 6.25 decreased dramatically after EGF treatment (orange box).

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 (Hyclone, cat# 1677-006), 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 5.

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

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 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-MEK1 (Millipore, cat# 07-641), 1:100, anti-phospho MEK1 (Thr292, Millipore, cat# 07-852), 1:50, anti-phospho MEK1 (Thr386) (Phospho Solution, cat# p180-386), 1:50 and anti-phospho MEK1 (S298) (Cell Signaling Technology, cat# CS9128), 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-MEK1 (Millipore, cat# 07-641)
Anti-phospho MEK1(Thr292) (Millipore, cat# 07-852)
Anti-phospho MEK1 (Thr386) (Phospho Solution, cat# p180-386)
Anti-phospho MEK1 (S298) (Cell Signaling Technology, cat# CS9128)

Other antibody suggestions

Anti-phospho MEK1 (Abcam, cat# 32088)

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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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