Cell Biosciences™

NanoPro™ Assay: Thioredoxin Loading Control

APPLICATION BRIEF No. 1007

SUMMARY

Primary Antibody: Anti-Trx1 (Cell Signaling Technology, cat# 2285)
Detection Antibody: Anti-Rabbit HRP (Cell Biosciences, p/n 040-656)

Thioredoxin acts as an antioxidant and is found in nearly all known organisms. It exists in two isoforms and presents a double peak around pI 4.6. The data presented show the utility of Thioredoxin as a loading control for EGF treated A549 and HeLa cells.

RESULTS

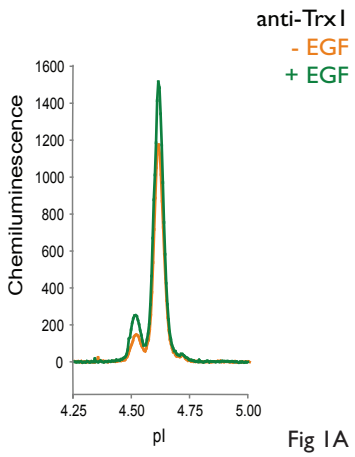

Fig 1A

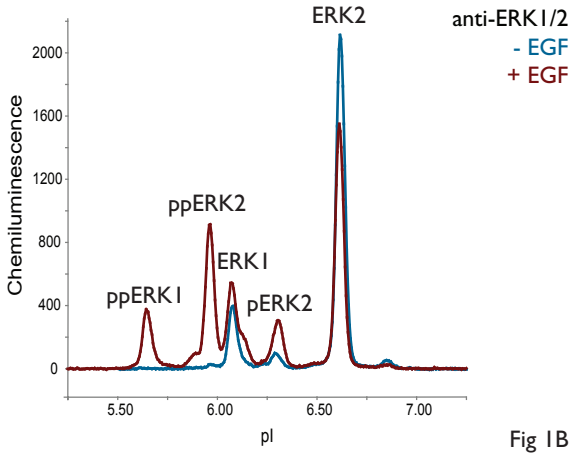

Fig 1B

Trx1 as a loading control in A549 cells

A549 cells were treated with 30 ng/mL epidermal growth factor (EGF) for 5 minutes. The EGF treatment caused induction of ERK1 and ERK2 phosphorylation (Fig 1B), but did not significantly affect Trx1 signals (Fig 1A).

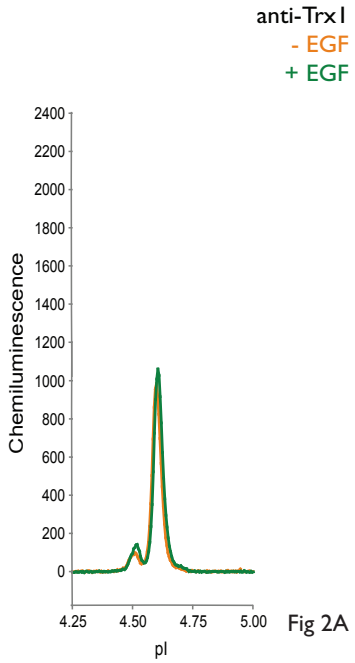

Fig 2A

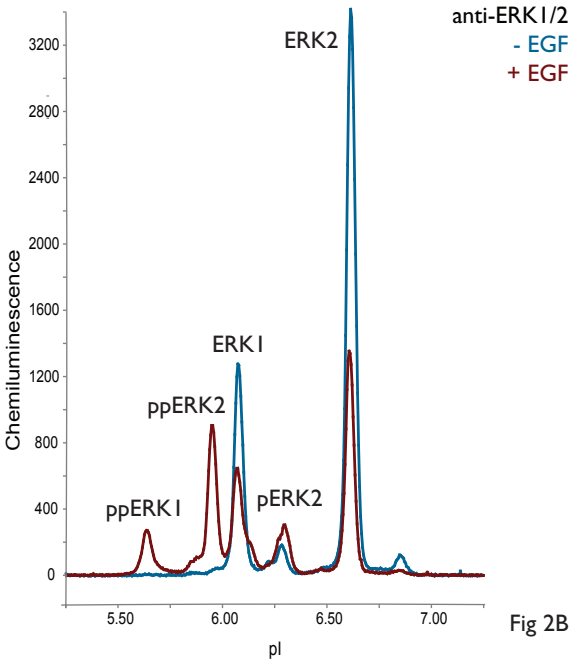

Fig 2B

Trx1 as a loading control in HeLa cells

HeLa cells were treated with 50 ng/mL EGF for 15 minutes. The EGF treatment caused induction of ERK1 and ERK2 phosphorylation (Fig 2B), but did not significantly affect Trx1 signals (Fig 2A).

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 (Hyclone, cat# SH30070.03) 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 DMEM without serum 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

Cell culture: A549 cells (ATCC, cat# CCL-185) were cultured in F-12K media (ATCC, cat# 30-2004) containing 10% FBS and 1x Penicillin/Streptomycin/Glutamine. Cells were split 1:5 every 3 days using 0.25% trypsin 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 F-12K without serum.

Treatment: 30 ng/mL EGF in F-12K without serum for 5 minutes at 37 °C, 5% CO_2.

Lysis buffer: Bicine/CHAPS Lysis Buffer plus 1x DMSO Inhibitor Mix and 1x Aqueous Inhibitor Mix.

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: 80% Premix 5-8 (Cell Biosciences Premix G1, p/n 040-327 or Premix G2, p/n 040-973) and 20% Premix 4-9 (Cell Biosciences Premix G1, p/n 040-319 or Premix G2, p/n 040-969)

pI standards: pI Standards 4.0 and 7.0 (Cell Biosciences, p/n 040-024 and p/n 040-031), 1:100

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

Primary antibody: Anti-Trx1 (Cell Signaling Technology, cat# 2285), 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 antibody

Anti-Thioredoxin1 (Cell Signaling Technology, cat# 2285)

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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 G1pI 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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