

NanoPro™ Assay: Heat Shock Protein 70 (Hsp70)

SUMMARY

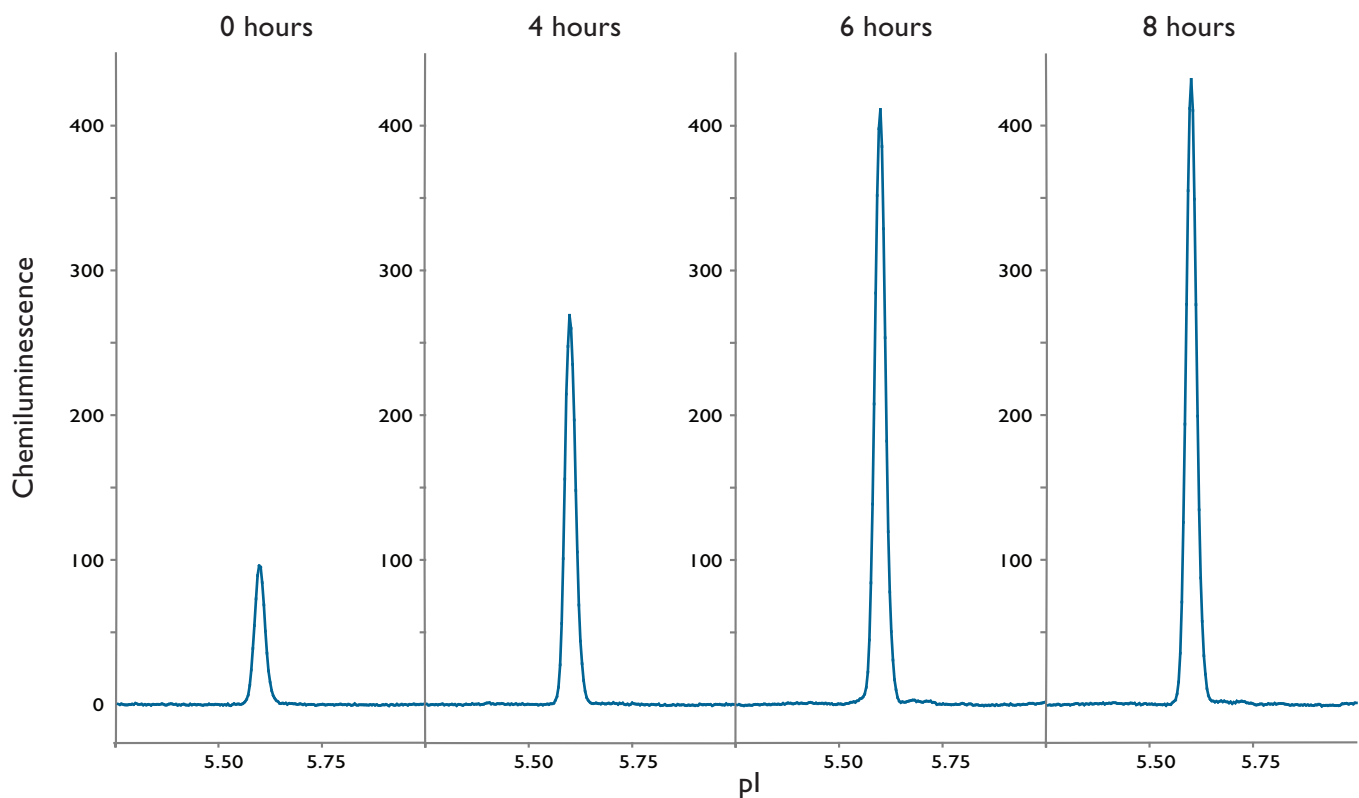
Primary Antibody: Anti-Hsp70 [W27] (Novus Biologicals, cat# NB600-571)

Detection Antibody: Anti-Mouse HRP (Cell Biosciences, p/n 040-655)

The 70 kDa heat shock proteins (Hsp70) are a family of ubiquitously expressed heat shock proteins. Proteins with similar structure exist in virtually all living organisms. The Hsp70s are an important part of the cell's machinery, and help to protect proteins from misfolding under stress. We show an increase of Hsp-70 expression in response to heat shock at 42 °C in A549 cells.

RESULTS

anti-Hsp70 [W27]



Strong increase in expression of Hsp70 after heat shock treatment

A549 cells were placed at 42 °C for 0, 4, 6, or 8 hours. An increase in Hsp70 signal was detected with increasing exposure to elevated temperatures.

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:** A549 cells (ATCC, cat# CCL-185) were cultured in F-12K media (ATCC, cat# 30-2004) 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 3.
- Treatment:** Cells were incubated for 4, 6, or 8 hours at 42 °C, 5% CO₂ while control remained at 37 °C, 5% CO₂.
- 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 × 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:** 0.003 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 Standards 4.92, 5.5, 7.0 (Cell Biosciences, p/n 040-027, p/n 040-028, p/n 040-031), 1:100
- Wash:** Wash Buffer (Cell Biosciences, p/n 040-654)
- Primary antibody:** Anti-Hsp70 [W27] (Novus Biologicals, cat# NB600-571), 1:500 in Antibody Diluent (Cell Biosciences, p/n 040-309)
- Detection antibody:** Anti-Mouse HRP (Cell Biosciences, p/n 040-655), 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 × 150 seconds (default)
- Primary antibody incubation:** 60 minutes
- Wash 2:** 2 × 150 seconds (default)
- Detection antibody incubation:** 60 minutes
- Wash 3:** 2 × 150 seconds (default)
- Chemiluminescence exposure:** 60, 120, and 240 seconds

Our favorite antibody

Anti-HSP-70 (Novus Biologicals, cat# NB600-571)

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 G1 and pl standards. | In a separate tube, mix Premix G2 and pl standards. |
| Step 3 | Mix equal parts of diluted lysate prepared in Step 1 with the Premix G1 + pl 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 + pl 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 μ L 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.