Cell Biosciences™

NanoPro™ Assay: Caspase 3

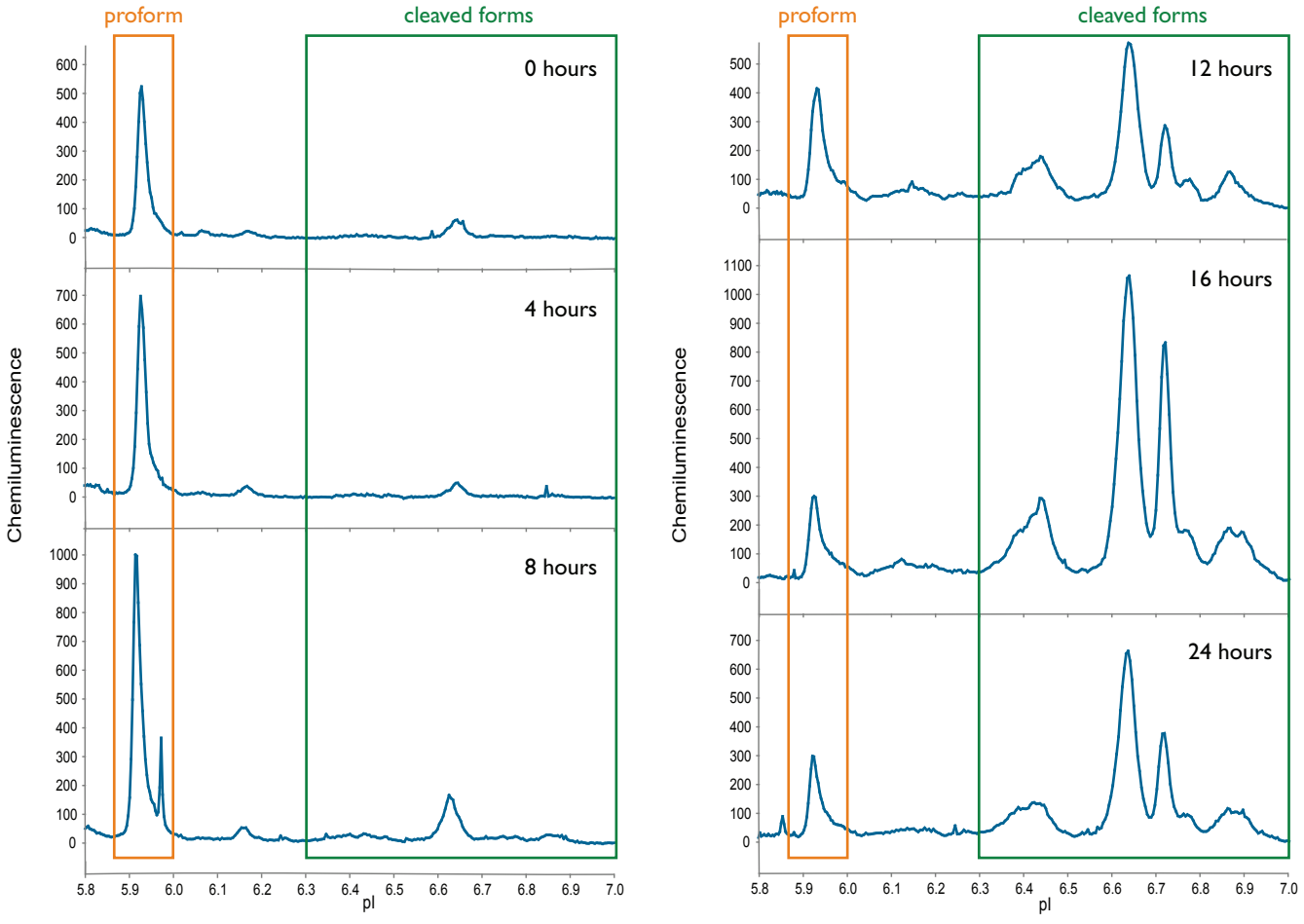
APPLICATION BRIEF No. 1010

SUMMARY

Primary Antibody: Anti-cleaved Caspase 3 (Asp175) (Cell Signaling Technology, cat# 9661)
Detection Antibody: Anti-Rabbit HRP (Cell Biosciences, p/n 040-656)

Caspases 3 exists as an inactive proenzyme that undergoes proteolytic processing at conserved aspartic residues to produce two subunits, large and small, that dimerize to form the active enzyme. Its activation is an important apoptosis marker. The data shows increased signal with antibodies specific to the Caspase 3 p17 subunit in response to apoptosis induction through prolonged treatment of K562 cells with Iminatib (aka Gleevec®) at an expected pI of around 6.3–6.5.

RESULTS



Time-dependent cleavage of pro-Caspase 3 in Imatinib-treated K562 cells

K562 cells were treated with 5 µM Imatinib for 0, 4, 8, 12, 16, and 24 hours. The pro-Caspase 3 peak labeled proform at pI 5.95 was identified through specific recognition by pro-Caspase 3 antibodies (Millipore, cat# 04-440 and cat# 05-654, and Cell Signaling Technology, cat# CS9662) (data not shown). The Caspase 3 fragment peaks labeled cleaved forms were induced by Imatinib treatment and recognized by specific anti-p17 antibodies. Interestingly, most antibodies described as anti-p17 specific also cross-react with the proform.

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: K562 cells (ATCC, cat# CCL-243) were cultured in RPMI 1640 media (Cellgro, cat# 10-041-CV) containing 10% FBS (Hyclone, cat# SH30070.03) and 1x Penicillin/Streptomycin/Glutamine (JRS Scientific, cat# 20020). Cells were split 1:5 every 3 days. Data shown from cells at passage 5.

Pre-treatment: Cells were starved for 20 hours before stimulation at 37 °C, 5% CO_2 in starvation medium containing RPMI 1640 without serum.

Treatment: 5 µM Imatinib Methanesulfonate Salt (LC Laboratories, cat# I-5508) in starvation medium for 0, 4, 8, 12, 16, or 24 hours 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: Collect cells by centrifugation (1000 x g, 5 minutes). Transfer cells to a 15-mL centrifuge tube, spin (1000 x g, 5 minutes) to pellet the cells. Aspirate media. Wash cell pellet with 1 mL of ice-cold PBS (Cellgro, cat# 21-031-CV). Transfer cells to a 1.5-mL centrifuge tube, spin (14,000 x g, 2 minutes). Aspirate wash. Keeping tube on ice, add 400 µL ice-cold lysis buffer to pellet, pipet up and down to resuspend. Incubate for an additional 30 minutes, rotating. Clarify by centrifugation (14,000 x g, 15 minutes). 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: 0.15 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 5-8 (nested) (Cell Biosciences Premix G1, p/n 040-643 or Premix G2, p/n 040-972)
pI standards: pI Standard Ladder 3 (Cell Biosciences, p/n 040-646)
Wash: Wash Buffer (Cell Biosciences, p/n 040-654)
Primary antibody: Anti-Cleaved Caspase 3 (Asp175) (Cell Signaling Technology, cat# 9661), 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: 120 seconds
Wash 1: 2 x 150 seconds (default)
Primary antibody incubation: 240 minutes
Wash 2: 2 x 150 seconds (default)
Detection antibody incubation: 60 minutes
Wash 3: 2 x 150 seconds (default)
Chemiluminescence exposure: 120, 240, and 480 seconds

Our favorite antibodies

Anti-Cleaved Caspase 3 (Asp175) (Cell Signaling Technology, cat# 9661)
Anti-Cleaved Caspase 3 (Asp175) (5A1E) (Cell Signaling Technology, cat# 9664)

Other antibody suggestions

Anti-Caspase 3 (pro-form) (Millipore, cat# 04-440)
Anti-Caspase 3 (Abcam, cat# ab2302)
Anti-Caspase 3 (Abcam, cat# ab77973)
Anti-Caspase 3 (Cell Signaling Technology, cat# 9662)
Anti-Caspase 3 clone 4-1-18 (Millipore, cat# 05-654)
Anti-Caspase 3 [E83-77] (Abcam, cat# ab32042)
Anti-Caspase 3 active (cleaved) form (Millipore, cat# ab3623)
Anti-Caspase 3 p17 (T-20) (Santa Cruz Biotechnology, cat# sc-22140)

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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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