NanoPro™ Assay: cIAP1

SUMMARY

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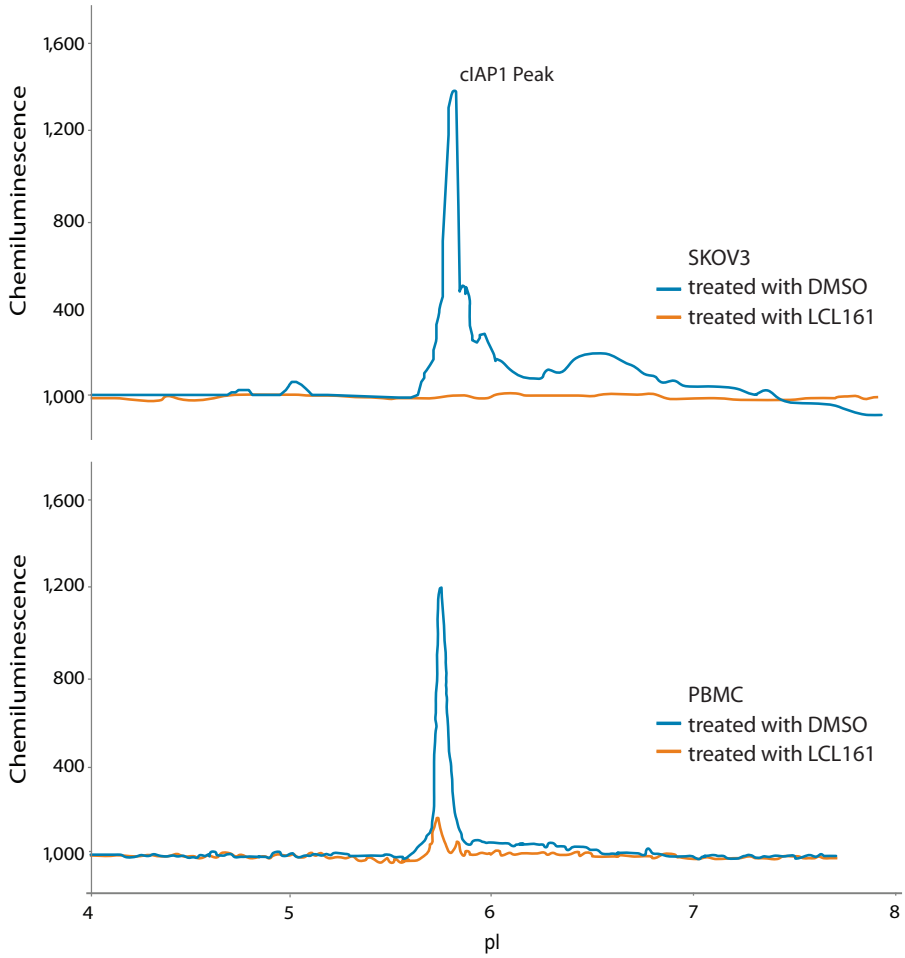
Primary Antibody: Goat Anti-cIAP1 (R&D systems, cat# AF8181)

Detection Antibody: Mouse Anti-Goat HRP (Thermo Fisher Scientific, cat# 31400)

cIAP1 is a member of the inhibitor of apoptosis (IAPs) family of proteins. It is up-regulated in several human cancers and plays an important role in tumor survival. cIAP1 functions to prevent cellular apoptosis by preventing the activation and/or inhibiting the function of different caspases.

RESULTS



SMAC- MIMETIC INHIBITOR, LCL161 TREATMENT INHIBITS CIAP1 LEVELS IN SKOV3 AND PBMC CELLS

SKOV3 cells (4×10^5/mL) and blood from healthy donors (8 mL) were treated with 2 µM LCL161 or DMSO for 2 hours. The anti-cIAP1 antibody recognized a peak at pI 5.77 in DMSO (blue trace). This peak was inhibited by LCL161 treatment (orange trace).

NOTE: Detection of the chemiluminescent signal produced is relative. Absolute units may vary depending on cell line, treatment and assay conditions.

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PROTOCOL

CELL PREPARATION

Cell culture:	SKOV3 cells are maintained in a humidified 5% CO_2 incubator at 37 °C. Cell lines are grown in RPMI1640 medium, penicillin/streptomycin and 10% fetal bovine serum.
Lysis buffer:	Bicine/CHAPS Lysis Buffer (ProteinSimple, p/n 040-764) plus 1x DMSO Inhibitor Mix (ProteinSimple, p/n 040-510) and 1x Aqueous Inhibitor Mix (ProteinSimple, p/n 040-482).
Lysis details:	Cells are washed with ice cold 1x PBS and lysed with 150 µL of Bicine CHAPs Lysis Buffer with Aqueous Inhibitor Mix and DMSO Inhibitor Mix for 30 min on ice. Lysates are cleared by centrifugation at 13,000 x g for 15 minutes at 4 °C and protein concentrations are determined by the Lowry method (Bio-Rad).
Storage:	–80 °C

PBMC PREPARATION

Sample collection:	Collect 8 mL of blood from healthy donors in CPT tubes (BD vaccutainer, Ref# 362753) and invert 8 – 10 times. Centrifuge the blood sample at room temperature in a horizontal rotor (swing-out head) at 1500 to 1800 RCF (Relative Centrifugal Force) for 20 minutes. After centrifugation, mononuclear cells and platelets will be in a whitish layer just under the plasma layer. Immediately after centrifugation, use a serological pipette to remove and discard as much of the plasma layer as possible without disturbing the white layer. Next, collect the white layer and transfer to a 15 mL conical tube with cap (BD cat# 352096). Add 10 mL of ice cold PBS and centrifuge at 1000 RCF for 10 minutes at 4 °C. Aspirate and discard the supernatant without disturbing the cell pellet. Add 0.5 mL of ice cold PBS to the pellet to resuspend and then transfer to a 1.5 mL eppendorf tube. Centrifuge the tube at 13000 x g for 5 minutes in a microfuge at 4 °C. Discard the supernatant without disturbing the pellet.
Lysis buffer:	Bicine/CHAPS Lysis Buffer (ProteinSimple, p/n 040-764) plus 1x DMSO Inhibitor Mix (ProteinSimple, p/n 040-510) and 1x Aqueous Inhibitor Mix (ProteinSimple, p/n 040-482).
Lysis details:	Add 100-150 µL of Bicine CHAPs Lysis Buffer with Aqueous Inhibitor Mix and DMSO Inhibitor Mix to the PBMC pellet. Pipette up and down until the pellet is resuspended. Incubate for 30 minutes on ice. Lysates are cleared by centrifugation at 13,000 x g for 15 minutes at 4 °C and protein concentrations are determined by the Lowry method (Bio-Rad).
Storage:	–80 °C

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ASSAY REAGENTS

NOTE: For specifics on sample preparation, please consult the addendum to this document.

Protein concentration:	0.1 mg/mL for cell lysates and 0.25 mg/mL for PBMCs (final concentration in capillary by Lowry method).
Sample Diluent:	Bicine/CHAPS Lysis Buffer plus 1x DMSO Inhibitor Mix
Ampholyte Premix:	Premix 3 – 10 (ProteinSimple Premix G1, p/n 040-806 or Premix G2, p/n 040-968)
pI Standards:	pI Standard Ladder 1 (ProteinSimple, p/n 040-644)
Wash:	Wash Buffer (ProteinSimple, p/n 040-654)
Primary antibody:	Goat Anti–cIAP1 (R&D systems, cat# AF8181), 5µg/mL
Detection antibody:	Mouse Anti–Goat HRP (Thermo Fisher Scientific, cat# 31400), 1:100 in Antibody Diluent (ProteinSimple, p/n 040-309)
Anolyte:	Phosphoric Acid, 10 mM (ProteinSimple, p/n 040-650)
Catholyte:	Sodium Hydroxide, 100 mM (ProteinSimple, p/n 040-651)
Luminol/Peroxide:	Mixed 1:1 (ProteinSimple, 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 proteinsimple.com, contact your ProteinSimple Field Applications Specialist or call Technical Support at (888) 607-9692.

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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:	40 seconds
Wash 1:	2 x 150 seconds (default)
Primary antibody incubation:	120 minutes
Wash 2:	2 x 150 seconds (default)
Detection antibody incubation:	30 minutes
Wash 3:	2 x 150 seconds (default)
Chemiluminescence exposure:	60, 120, 240, 480 and 960 seconds

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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 µL per sample well is recommended. Centrifugation of the sample plate (3000 x g, 10 minutes) is required.

For further assistance, please contact your ProteinSimple Field Applications Specialist or Technical Support at (888) 607-9692.

OUR FAVORITE ANTIBODIES

Goat Anti – cIAP1 (R&D systems, cat# AF8181).

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