NanoPro™ Assay: Acetylation

SUMMARY

Primary Antibody: pan AcLysine (Cell Signaling, cat# 9441)
Detection Antibody: Anti-Mouse HRP and Anti-Rabbit HRP (Protein Simple, p/n 040-655 and p/n 040-657)

Acetylation is a common post-translation modification typically observed on chromatin proteins as well as other regulatory proteins and metabolic enzymes. The reaction is catalyzed by N-terminal acetyltransferases, occurs predominantly during protein synthesis and appears to be irreversible. Pan-Ac-Lysine antibodies are widely used for detection of acetylation of imunoprecipitated proteins when no specific antibodies are available.

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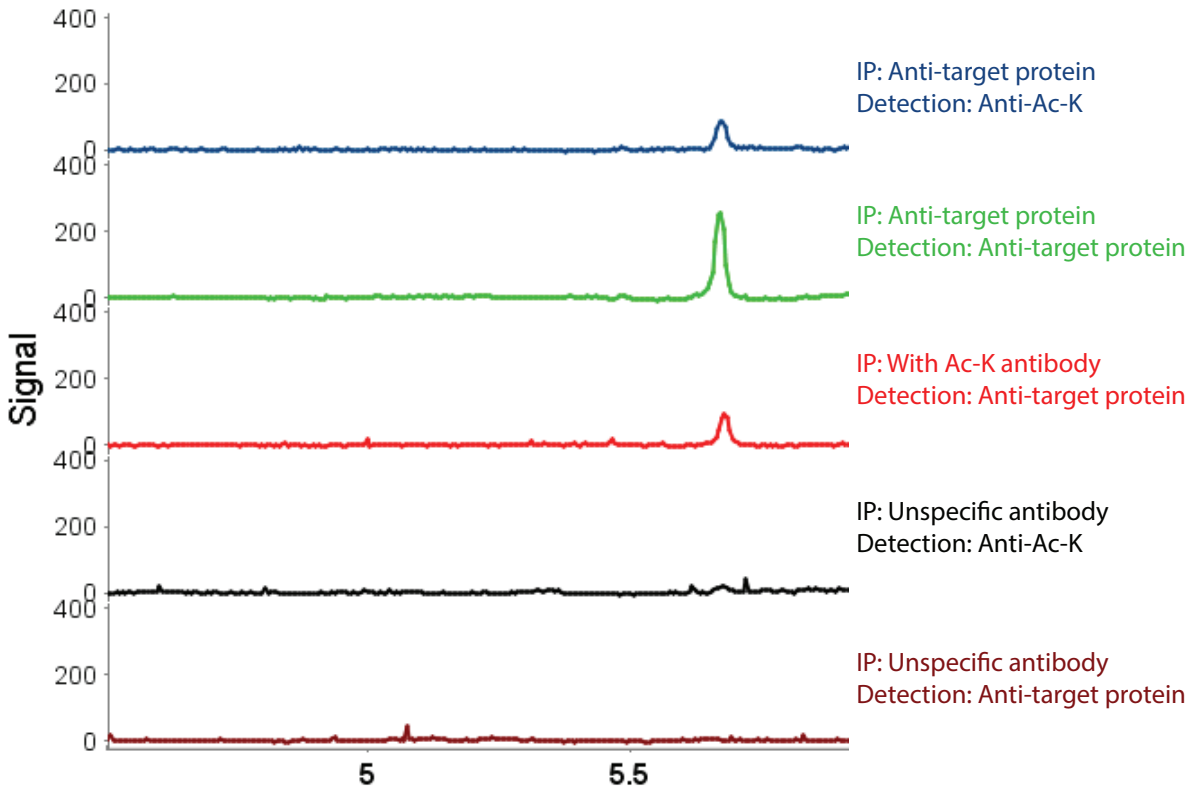
RESULTS



CHARGE PROFILE OF IMMUNOPRECIPITATED ACETYLATED PROTEINS

Immunoprecipitated protein detection in RIPA cell extracts by specific antibodies against target protein (green) and acetyl-lysine antibodies (blue). Presence of acetylated lysine residues was confirmed by detection of the target protein in the immunoprecipitates using acetyl-lysine antibodies (red). Control samples immunoprecipitated using a non-target antibody did not have the peak in this pI range when probed with either anti-Ac-K (black) or anti-target antibodies (brown).

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PROTOCOL

PROCEDURE

Lysates were generated under optimized conditions for specific targets using either Bicine/CHAPS Buffer Lysis Kit (ProteinSimple, p/n CBS403) or RIPA Buffer Lysis Kit (ProteinSimple, p/n CBS401) according to the product insert protocols.

Step 1:	Immunoprecipitations were performed using either specific target protein antibody, pan AcLysine antibody or an antibody non-specific to the target protein of interest. Concentrations of antibodies were 1:100. Lysates were pre-cleared (incubation with beads for 1 hour at 4 °C), incubated with antibody overnight at 4°C and the complexes were precipitated on beads 2-4 hours at 4 °C. Protein G Magnetic Beads are from Millipore (cat #LSKMAGG10).
Step 2:	Wash with RIPA Buffer (ProteinSimple, p/n 040-483).
Step 3:	Elute with 9 M urea, 40 mM DTT or 50 mM glycine, pH 2.5, 10 mM DTT for 5 minutes at room temperature with two washes.
Step 4:	Dilute urea samples with Bicine/CHAPS Buffer (ProteinSimple, p/n 040-764) to reduce urea concentration in the capillary to less than 2 M.

NOTE: The antibody used on the NanoPro1000 and the antibody used for immunoprecipitation should be from 2 different species.

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ASSAY REAGENTS

Sample Diluent:	Bicine/CHAPS Lysis Buffer (ProteinSimple, p/n 040-764) plus 1x DMSO Inhibitor Mix (ProteinSimple, p/n 040-510)
Ampholyte Premix:	50% Premix G2, pH 5-8 (ProteinSimple, p/n 040-972) plus 50% Premix G2, pH 3-10 (ProteinSimple, p/n 040-968)
pI Standards:	pI Standard Ladder 1 (ProteinSimple, p/n 040-644) supplemented with pI 4.4 standard (ProteinSimple, p/n 040-026)
Wash:	Wash Buffer (ProteinSimple, p/n 040-654)
Primary antibody:	pan AcLysine (Cell Signaling, cat #9441) diluted 1:50 in Antibody Diluent (ProteinSimple, p/n 040-309)
Detection antibody:	Goat Anti-Mouse HRP (ProteinSimple, p/n 040-655) and Goat Anti-Rabbit HRP (ProteinSimple, p/n 040-657), 1:100 in Antibody Diluent
Anolyte:	Phosphoric Acid, 10 mM (ProteinSimple, p/n 040-337)
Catholyte:	Sodium Hydroxide, 100 mM (ProteinSimple, p/n 040-338)
Luminol/Peroxide xDR:	Mixed 1:1 (ProteinSimple, p/n 041-084 and p/n 040-652)

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.

ASSAY CONDITIONS

System:	NanoPro 1000
Sample Loading Time:	25 seconds (Premix G2)
Focus Conditions:	60000 µW, 40 minutes (Premix G2)
Immobilization:	100 seconds
Wash 1:	2 x 150 seconds (default)
Primary antibody incubation:	120 minutes
Wash 2:	2 x 150 seconds (default)
Detection antibody incubation:	60 minutes
Wash 3:	2 x 150 seconds (default)
Chemiluminescence exposure:	30, 60, 120, 240, 480 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.

PREMIX G1 PROCEDURE	
Step 1	Dilute lysate with sample diluent to 0.1 mg/mL.
Step 2	In a separate tube, mix Premix G1 and pI standards.
Step 3	Mix equal parts of diluted lysate prepared in Step 1 with Premix G1 + pI Standards prepared in Step 2 (1:1 ratio) to create final protein concentration of 0.05 mg/mL.

PREMIX G2 PROCEDURE	
Step 1	Dilute lysate with sample diluent to 0.2 mg/mL.
Step 2	In a separate tube, mix Premix G2 and pI standards.
Step 3	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.
NOTE: 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.

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