NanoPro™ Assay: Histone

SUMMARY

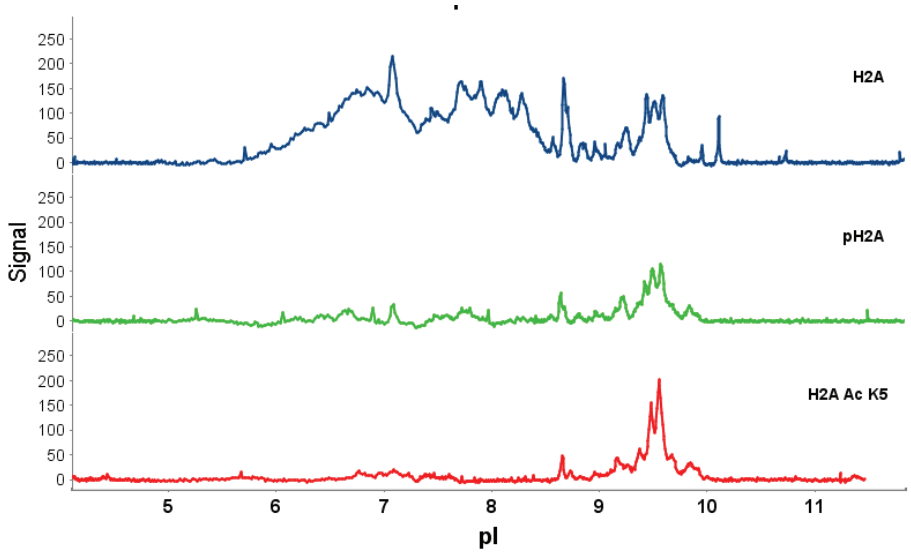
Primary Antibody: Histone H2A Antibody (Cell Signaling, cat #2578), Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb (Cell Signaling, cat #9718), Acetyl-Histone H2A (Lys5) Antibody (Cell Signaling, cat #2576)
Detection Antibody: Goat Anti-Rabbit HRP (Protein Simple, p/n 040-657)

Modulation of chromatin structure plays an important role in the regulation of transcription in eukaryotes. The nucleosome, made up of DNA wound around eight core histone proteins (two each of H2A, H2B, H3, and H4), is the primary building block of chromatin (1). The amino-terminal tails of core histones undergo various post-translational modifications, including acetylation, phosphorylation, methylation, and ubiquitination (2-5). These modifications occur in response to various stimuli and have a direct effect on the accessibility of chromatin to transcription factors and, therefore, gene expression (6). In most species, histone H2B is primarily acetylated at Lys5, 12, 15, and 20 (4,7). Histone H3 is primarily acetylated at Lys9, 14, 18, 23, 27, and 56. Acetylation of H3 at Lys9 appears to have a dominant role in histone deposition and chromatin assembly in some organisms (2,3). Phosphorylation at Ser10, Ser28, and Thr11 of histone H3 is tightly correlated with chromosome condensation during both mitosis and meiosis (8-10). Phosphorylation at Thr3 of histone H3 is highly conserved among many species and is catalyzed by the kinase haspin. Immunostaining with phospho-specific antibodies in mammalian cells reveals mitotic phosphorylation at Thr3 of H3 in prophase and its dephosphorylation during anaphase (11).

1) Workman, J.L. and Kingston, R.E. (1998) Annu Rev Biochem 67, 545-79. 2) Hansen, J.C. et al. (1998) Biochemistry 37, 17637-41.
3) Strahl, B.D. and Allis, C.D. (2000) Nature 403, 41-5. 4) Cheung, P. et al. (2000) Cell 103, 263-71. 5) Bernstein, B.E. and Schreiber, S.L. (2002) Chem Biol 9, 1167-73. 6) Jaskelioff, M. and Peterson, C.L. (2003) Nat Cell Biol 5, 395-9. 7) Thorne, A.W. et al. (1990) Eur J Biochem 193, 701-13. 8) Hendzel, M.J. et al. (1997) Chromosoma 106, 348-60. 9) Goto, H. et al. (1999) J Biol Chem 274, 25543-9. 10) Preuss, U. et al. (2003) Nucleic Acids Res 31, 878-85. 11) Dai, J. et al. (2005) Genes Dev 19, 472-88. 12) Molecular and Cellular Biology, Oct. 2007, p. 7028–7040 Vol. 27, No. 20.

Authors: Irina Tikhanovich PhD and Steven Weinman MD, PhD, University of Kansas Medical Center, US (itikhanovich@kumc.edu)

RESULTS



HISTONE H2A DETECTION IN HEPATOMA CELLS

Histone H2A isoforms in the nuclear extracts of Huh 7.5 cells recognized by total H2A atibody (blue trace), phospho-Histone H2A.X (Ser139) antibody (green trace) and acetyl-Histone H2A (Lys5) Antibody (red trace).

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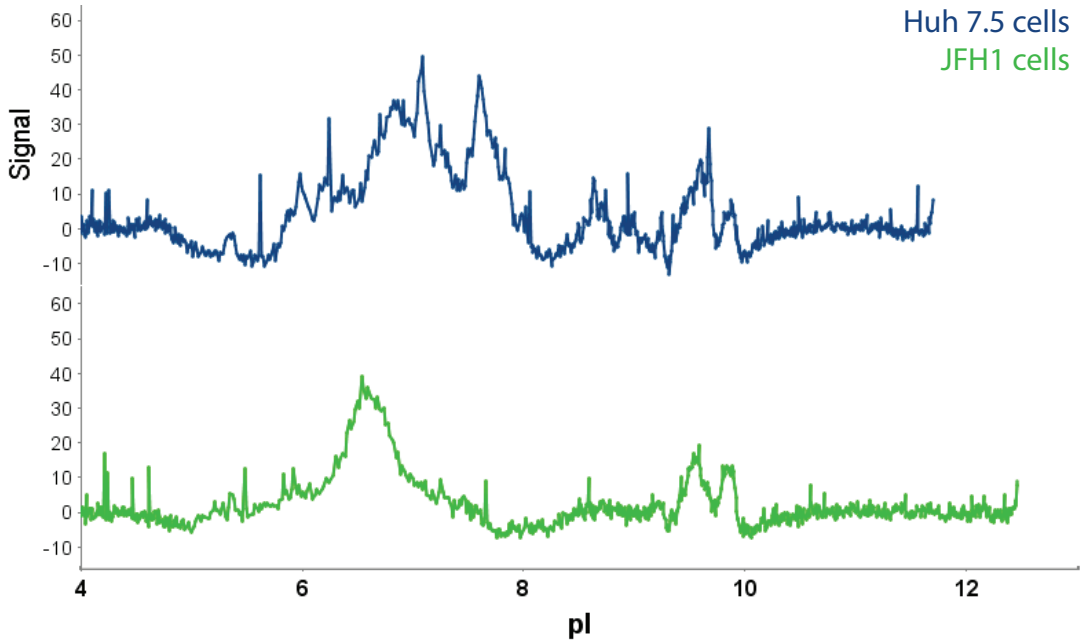

HISTONE H2A DETECTION IN NUCLEAR EXTRACTS OF HUH 7.5 CELLS

Histone H2A isoforms in the nuclear extracts of Huh 7.5 cells mock infected (blue trace) or JFH1 infected (green trace) recognized by total H2A antibody. Cluster of peaks in the region of pI 7.1-9.0 are greatly diminished by the infection.

PROTOCOL

CELL PREPARATION

Cell culture:	Huh7.5 cells (obtained from Dr. Charles Rice) were maintained in Dulbecco's Modified Eagle's Medium (Invitrogen, Carlsbad, CA) containing 10% FBS, 50 U/mL penicillin and 50 mg/mL streptomycin.
Treatment:	Huh7.5 cells (2x106) were seeded onto T-175 flasks and infected the following day with HCV (Japanese Fulminant Hepatitis 1 (JFH1) at a multiplicity of infection of 0.5-1.0.
Lysis buffer:	Hypotonic Lysis Buffer A (10 mM Hepes, 10 mM KCl, 1.5 mM $MgCl_2$, 0.34 M sucrose, 10% glycerol, 1 mM DTT, 5 mM Na Butyrate, 0.6 % CHAPS), Hypertonic Lysis Buffer B (20 mM Hepes, 1.5 mM $MgCl_2$, 0.42 M NaCl, 0.2 mM EDTA, 25% Glycerol, 1 mM DTT, 1:100 protease and phosphatase inhibitors).
Lysis details:	Wash the cell pellet 2 times with ice cold 1X PBS at 3000 rpm for 5 minutes at 4 °C. After 2nd spin, add 2 mL of Buffer A, mix gently and leave on ice for 5 minutes. Next, spin at 3000 rpm for 5 minutes. Take the supernatant into a fresh 15-mL tube and centrifuge at 14000 x *g* for 20 minutes. Take the supernatant into a fresh tube, aliquot and store in -80 °C. To the pellet, add 2 mL Buffer B, keep on ice for 5 minutes and then centrifuge at 3000 rpm for 5 minutes. The resulting supernatant is the nuclear extract. To the pellet, add Buffer B, mix, and keep on ice for 20-30 minutes. Now sonicate for 12 -15 seconds (based on the pellet size). Keep sample on ice for 2 minutes, then centrifuge at 14000 x *g* for 20 minutes at 4 °C. Take the supernatant into a fresh tube, aliquot and proceed for protein estimation of both cytoplasmic and nuclear lysates.
Storage:	–80 °C

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ASSAY REAGENTS

Protein concentration:	0.1 mg/mL final in capillary by BCA assay
Sample Diluent:	Bicine/CHAPS Lysis Buffer (ProteinSimple, p/n 040-764) plus 1x DMSO Inhibitor Mix (ProteinSimple, p/n 040-510)
Ampholyte Premix:	G2 Premix 3-10 (ProteinSimple, p/n 040-968) plus 1-1.5% TEMED (BioRad, cat #161-0800)
pI Standards:	pI Standard Ladder 1 (ProteinSimple, p/n 040-644) supplemented with pI 8.4 and 9.7 standards (Protein Simple, p/n 041-036 and 040-970)
Procedure:	Step 1) Dilute lysate to 0.2 mg/mL with sample diluents. Step 2) In a separate tube mix ampholyte premix G2 and pI standards. Step 3) Mix step 1 and step 2 at 1:4 to create final protein concentration.
Wash:	Wash Buffer (ProteinSimple, p/n 040-654)
Primary antibody:	Histone H2A Antibody (Cell Signaling, cat #2578), Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb (Cell Signaling, cat #9718), Acetyl-Histone H2A (Lys5) Antibody (Cell Signaling, cat #2576); Antibodies diluted 1:50 in Antibody Diluent.
Detection antibody:	Anti-Rabbit HRP (Protein Simple, 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 (G2 Premix)
Focus Conditions:	60000 µW, 40 minutes (G2 Premix)
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:	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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Toll-free: (888) 607-9692
Tel: (408) 510-5500
Fax: (408) 510-5599
orders@proteinsimple.com
proteinsimple.com