#12784 Store at -20°C

Nucleus and Nuclear Envelope-Associated Marker Proteins Antibody Sampler Kit

☑ 1 Kit
(7 x 40 µl)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
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Support ■ 877-678-TECH (8324)
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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
ESET (C1C12) Rabbit mAb	2196	40 µl	180 kDa	Rabbit IgG
Fibrillarin (C13C3) Rabbit mAb	2639	40 µl	37 kDa	Rabbit IgG
Histone H2A.Z Antibody	2718	40 µl	14 kDa	Rabbit
Histone H3 (D1H2) XP® Rabbit mAb	4499	40 µl	17 kDa	Rabbit IgG
Lamin A/C (4C11) Mouse mAb	4777	40 µl	63, 74 kDa	Mouse IgG2a
LSD1 (C69G12) Rabbit mAb	2184	40 µl	110 kDa	Rabbit IgG
NUP98 (C39A3) Rabbit mAb	2598	40 µl	98 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat
Anti-mouse IgG, HRP-linked Antibody	7076	100 µl		Horse

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibodies.*

Recommended Antibody Dilutions:

Western blotting	1:1000
#4499, #4777	1:2000

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Description: The Nucleus and Nuclear Envelope-Associated Marker Proteins Antibody Sampler Kit provides an economical means to evaluate relevant nuclear proteins. This kit contains enough primary antibody to perform at least four western blots per primary antibody.

Background: The Nucleus and Nuclear Envelope-Associated Marker Proteins Antibody Sampler Kit contains a variety of antibodies directed against established nuclear proteins (1). Histone H3 and histone H2A.Z are histone family members and components of nucleosomes, the primary building block of chromatin made up of DNA wound around eight core histone proteins. The amino-terminal tails of core histones undergo various post-translational modifications and have a direct effect on the accessibility of chromatin to transcription factors and, therefore, gene expression (2). ESET histone methyltransferase (3) and LSD1 histone demethylase (4) are both regulators of histone methylation and are chromatin-associated. Both NUP98 (5) and lamins (6) are located within the nuclear envelope (also known as the nuclear membrane). NUP98 is a component of the nuclear pore complex. Lamin A and lamin C are fibrous proteins contributing to nuclear structural and transcriptional regulation. Finally, fibrillarin (7) is located in fibrillar regions and Cajal bodies of nucleoli, where it functions to regulate RNA transcription and pre-rRNA processing.

Specificity/Sensitivity: Each antibody in the Nucleus and Nuclear Envelope-Associated Marker Proteins Antibody Sampler Kit recognizes total endogenous levels of the specific target protein.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to the carboxy terminus of the human ESET protein, residues surrounding Thr298 of human fibrillarin, the carboxy terminus of the human histone H3 protein, a recombinant fragment of human lamin A protein, residues near the amino-terminus of human LSD1 protein, or residues surrounding Pro671 of human NUP98. Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to the carboxy terminus of human histone H2A.Z. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

Background References:

(1) Workman, J.L. and Kingston, R.E. (1998) *Annu Rev Biochem* 67, 545-79.

(2) Jin, J. et al. (2005) *Trends Biochem Sci* 30, 680-7.

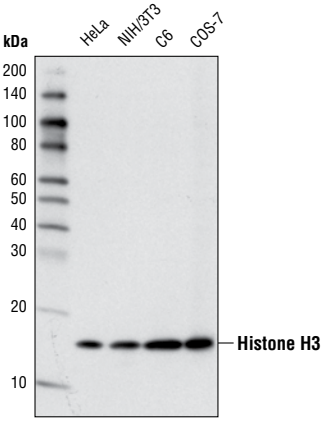
(3) Yang, L. et al. (2002) *Oncogene* 21, 148-52.

(4) Shi, Y. et al. (2004) *Cell* 119, 941-53.

(5) Fontoura, B.M. et al. (1999) *J Cell Biol* 144, 1097-112.

(6) Gruenbaum, Y. et al. (2000) *J Struct Biol* 129, 313-23.

(7) Tollervey, D. et al. (1993) *Cell* 72, 443-57.

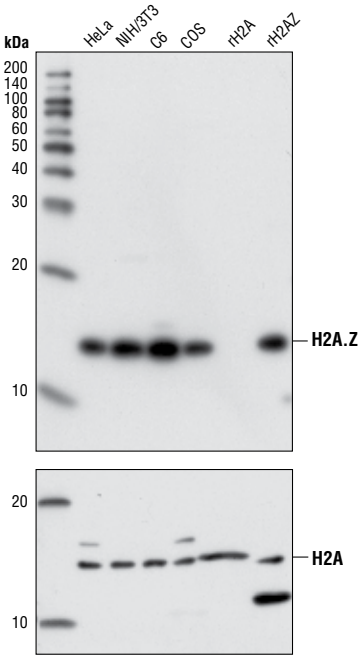


Western blot analysis of extracts from various cell lines using ***Histone H3 (D1H2) XP® Rabbit mAb #4499****.*

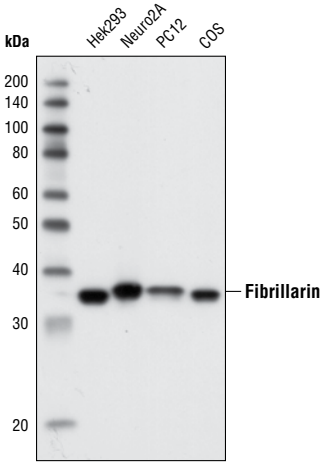
U.S. Patent No. 5,675,063

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

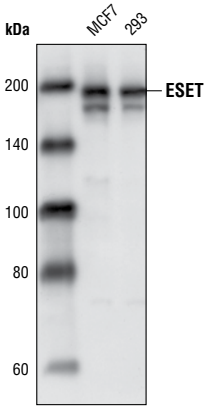
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



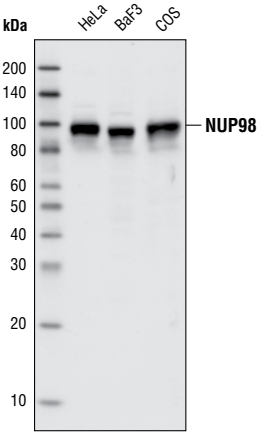
Western blot analysis of extracts from various cell lines, in addition to 10 ng of recombinant H2A (rH2A) and H2A.Z (rH2A.Z) protein, using ***Histone H2A.Z Antibody #2718*** *(upper) and Histone H2A Antibody II #2578 (lower).*



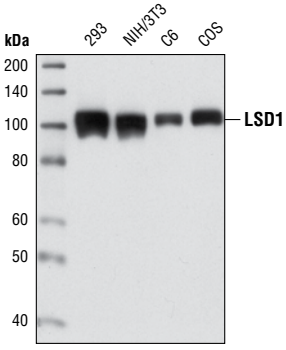
Western blot analysis of extracts from various cell lines using ***Fibrillarin (C13C3) Antibody #2639****.*



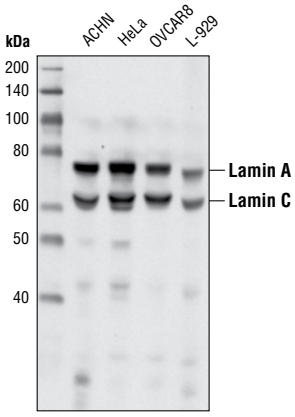
Western blot analysis of extracts from MCF7 and 293 cells using ***ESET (C1C12) Rabbit mAb #2196****.*



Western blot analysis of extracts from various cell lines using ***NUP98 (C39A3) Rabbit mAb #2598****.*



Western blot analysis of extracts from various cell lines using ***LSD1 (C69G12) Rabbit mAb #2184****.*



Western blot analysis of extracts from various cell lines using ***Lamin A/C (4C11) Mouse mAb #4777****.*

#12784

Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.