#9927 Store at –20°C

Acetyl-Histone H3 Antibody Sampler Kit

✓ 1 Kit
(6 x 40 µl)

Cell Signaling TECHNOLOGY®

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Support ■ 877-678-TECH (8324)
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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Products Included	Product #	Quantity	Mol. Wt.	Source
Acetyl-Histone H3 (Lys9) (C5B11) Rabbit mAb	9649	40 µl	17 kDa	Rabbit IgG
Acetyl-Histone H3 (Lys14) Antibody	4318	40 µl	17 kDa	Rabbit IgG
Acetyl-Histone H3 (Lys18) Antibody	9675	40 µl	kDa	Rabbit IgG
Acetyl-Histone H3 (Lys27) Antibody	4353	40 µl	17 kDa	Rabbit IgG
Acetyl-Histone H3 (Lys56) Antibody	4243	40 µl	kDa	Rabbit IgG
Histone H3 (D1H2) XP™ Rabbit mAb	4499	40 µl	17 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

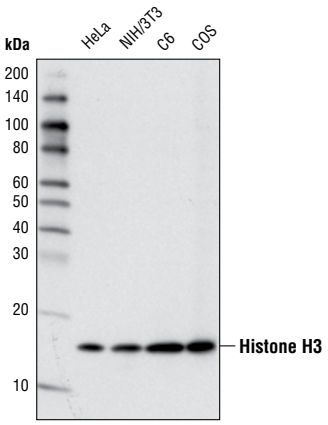
See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The Acetyl-Histone H3 Antibody Sampler Kitprovides a fast and economical means of evaluating the acetylation sites on Histone H3. The kit contains enough primary and secondary antibodies to perform four Western mini-blot experiments.

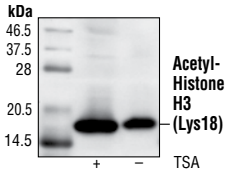
Background: Modulation of chromatin structure plays an important role in the regulation of transcription in eukaryotes. The nucleosome, made up of four core histone proteins (H2A, H2B, H3 and H4), is the primary building block of chromatin (1). The amino-terminal tails of core histones undergo various 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, on 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 and 23 (2,3). Acetylation 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 of 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 of H3 Thr3 in prophase and its dephosphorylation during anaphase (11).

Specificity/Sensitivity: All antibodies in the Acetyl-Histone H3 Antibody Sampler Kit recognize histone H3 only when modified at the indicated site.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with synthetic acetylated peptides (KLH-coupled) corresponding to residues surrounding Lys9, Lys14, Lys18, Lys27, or Lys56 of human Histone H3. Antibodies are purified by protein A and peptide affinity chromatography.



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



Western blot analysis of extracts from NIH/3T3 cells with or without TSA treatment, using ***Acetyl-Histone H3 (Lys18) Antibody #9675****.*

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

Recommended Antibody Dilutions:
Western blotting 1:1000
See www.cellsignal.com for individual component dilutions and additional application protocols.

Background References:

(1) Workman, J.L. and Kingston, R.E. (1998) *Annu. Rev. Biochem.* 67, 545–579.
(2) Hansen, J.C. et al. (1998) *Biochemistry* 37, 17637–17641.
(3) Strahl, B.D. and Allis, C.D. (2000) *Nature* 403, 41–45.
(4) Cheung, P. et al. (2000) *Cell* 103, 263–271.
(5) Bernstein, B.E. and Schreiber, S.L. (2002) *Chem. Biol.* 9, 1167–1173.
(6) Jaskelioff, M. and Peterson, C.L. (2003) *Nat. Cell Biol.* 5, 395–399.
(7) Thorne, A.W. et al. (1990) *Eur. J. Biochem.* 193, 701–713.
(8) Hendzel, M.J. et al. (1997) *Chromosoma* 106, 348–360.
(9) Goto, H. et al. (1999) *J. Biol. Chem.* 274, 25543–25549.
(10) Preuss, U. et al. (2003) *Nucleic Acids Res.* 31, 878–885.
(11) Dai, J. et al. (2005) *Genes Dev.* 19, 472–488.

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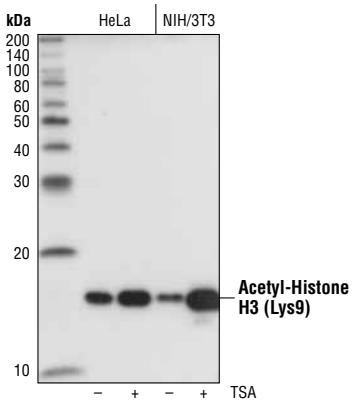
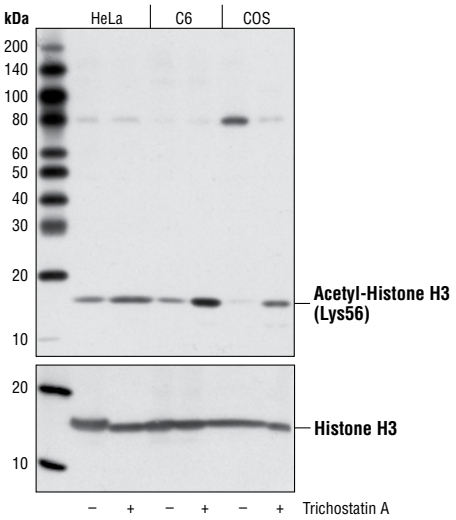
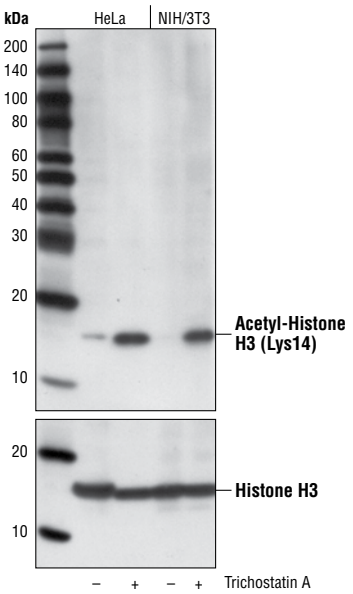
Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



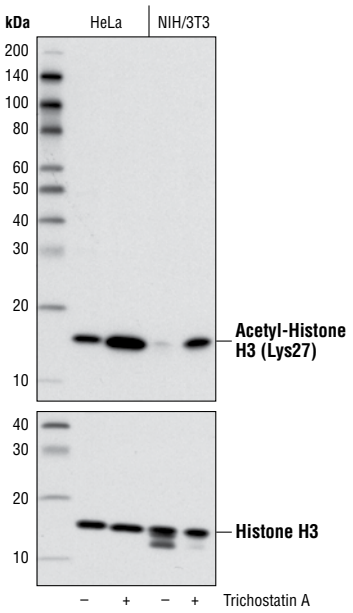
Western blot analysis of lysates from HeLa and NIH/3T3 cells, untreated or TSA-treated (400 nM for 18 hours) using ***Acetyl-Histone H3 (Lys9) (C5B11) Rabbit mAb #9649.***



Western blot analysis of extracts from HeLa, C6 and COS cells, untreated or treated with Trichostatin A (TSA) #9950 (400 nM for 18 h), using ***Acetyl-Histone H3 (Lys56) Antibody #4243*** *(upper) and Histone H3 Antibody #9715 (lower).*



Western blot analysis of extracts from HeLa and NIH/3T3 cells, untreated or treated with Trichostatin A #9950 (400 nM for 18 h), using ***Acetyl-Histone H3 (Lys14) Antibody #4318*** *(upper) and Histone H3 Antibody #9715 (lower).*



Western blot analysis of extracts from HeLa and NIH/3T3 cells, untreated or treated with Trichostatin A #9950 (400 nM for 18 h), using ***Acetyl-Histone H3 (Lys27) Antibody #4353*** *(upper) and Histone H3 Antibody #9715 (lower).*

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Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

1. 1X Phosphate Buffered Saline (PBS)
2. **1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
10. **Phototope®-HRP Western Blot Detection System** #7071**:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

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 per plate of 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 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

8. Electrotransfer to nitrocellulose or PVDF membrane.

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.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO® substrate can be further diluted if signal response is too fast.

2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.

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