

Acetyl-Histone H3 Antibody Sampler Kit

✓ 1 Kit
 (6 x 20 µl)



Orders ■ 877-616-CELL (2355)
 orders@cellsignal.com
Support ■ 877-678-TECH (8324)
 info@cellsignal.com
Web ■ www.cellsignal.com

rev. 06/16

For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Source
Acetyl-Histone H3 (Lys9) (C5B11) Rabbit mAb	9649	20 µl	17 kDa	Rabbit IgG
Acetyl-Histone H3 (Lys27) (D5E4) XP® Rabbit mAb	8173	20 µl	17 kDa	Rabbit IgG
Acetyl-Histone H3 (Lys18) (D8Z5H) Rabbit mAb	13998	20 µl	17 kDa	Rabbit IgG
Acetyl-Histone H3 (Lys14) (D4B9) Rabbit mAb	7627	20 µl	17 kDa	Rabbit IgG
Acetyl-Histone H3 (Lys56) Antibody	4243	20 µl	17 kDa	Rabbit IgG
Histone H3 (D1H2) XP® Rabbit mAb	4499	20 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 Kit provides an economical means of evaluating the acetylation sites on Histone H3. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

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). Phosphoryla-

tion 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 peptide corresponding to residue Lys56 of human Histone H3. Antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding acetylated Lys18 of human H3 protein, acetylated Lys27 of human histone H3 protein, acetylated Lys14 of human Histone H3 protein, or the amino terminus of histone H3 in which Lys9 is acetylated.

Background References:

(1) Workman, J.L. and Kingston, R.E. (1998) *Annu. Rev. Biochem.* 67, 545-579.

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

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

(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.

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 Ce—C. elegans Hr—horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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.

- 20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH₂O, mix.
- 10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH₂O, mix.
- 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 dH₂O.
- 10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH₂O, mix.
- 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 dH₂O, mix.
- 10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH₂O, mix.
- Nonfat Dry Milk:** (#9999)
- 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.
- Wash Buffer:** (#9997) 1X TBST
- Bovine Serum Albumin (BSA):** (#9998)
- 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.
- Biotinylated Protein Ladder Detection Pack:** (#7727)
- Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
- Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
- Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
- Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- 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.
- Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
- Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
- Microcentrifuge for 5 min.
- 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.
- Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

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

II. Primary Antibody Incubation

- 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.
- Wash three times for 5 min each with 15 ml of TBST.
- 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.
- Wash three times for 5 min each with 15 ml of TBST.
- Proceed with detection (Section D).

D. Detection of Proteins

- 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.
- 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.