Acetylated-Lysine Antibody

#9441 Store at –20°C

☐ Small 100 µl (10 western blots)
☐ Large 300 µl (30 western blots)

Cell Signaling TECHNOLOGY®

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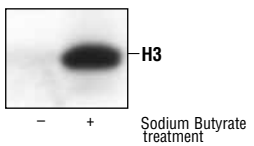
For Research Use Only. Not For Use In Diagnostic Procedures.

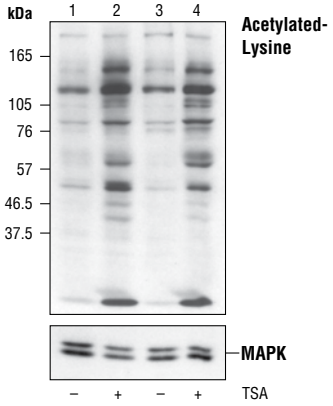
Applications	Species Cross-Reactivity*	Source	Motif
W, IP, IHC-P, IF-IC, E-P, ChIP	All	Rabbit**	XXX(Kac)XXX
Endogenous			

Background: Acetylation of lysine, like phosphorylation of serine, threonine or tyrosine, is an important reversible modification controlling protein activity. The conserved amino-terminal domains of the four core histones (H2A, H2B, H3, and H4) contain lysines that are acetylated by histone acetyltransferases (HATs) and deacetylated by histone deacetylases (HDACs) (1). Signaling resulting in acetylation/deacetylation of histones, transcription factors, and other proteins affects a diverse array of cellular processes including chromatin structure and gene activity, cell growth, differentiation, and apoptosis (2-6). Recent proteomic surveys suggest that acetylation of lysine residues may be a widespread and important form of posttranslational protein modification that affects thousands of proteins involved in control of cell cycle and metabolism, longevity, actin polymerization, and nuclear transport (7,8). The regulation of protein acetylation status is impaired in cancer and polyglutamine diseases (9), and HDACs have become promising targets for anti-cancer drugs currently in development (10).

Specificity/Sensitivity: Acetylated-Lysine Antibody detects proteins posttranslationally modified by acetylation on the epsilon-amine groups of lysine residues. The antibody recognizes acetylated lysine in a wide range of sequence contexts. It has been demonstrated to recognize acetylated histones, p53, CBP, PCAF and chemically acetylated BSA. The antibody has been shown to react with as little as 0.04 ng of chemically acetylated BSA while not recognizing up to 25 µg of nonacetylated BSA. (U.S. Patent No's.: 6,441,140; 6,982,318; 7,259,022; 7,344,714; U.S.S.N. 11,484,485; and all foreign equivalents.)

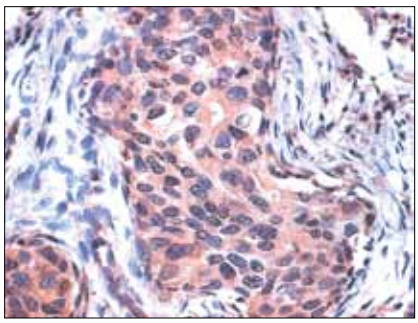


Western blot analysis of extracts from NIH/3T3 cells, untreated or sodium butyrate-treated (5 mM for 24 hours), showing an increase in histone acetylation, using Acetylated-Lysine Antibody.



Western blot analysis of extracts from COS cells, untreated or TSA-treated, grown in 10% FBS (lanes 1 and 2) or serum starved for 18 hours (lanes 3 and 4), using Acetylated-Lysine Antibody (upper) or p44/42 MAP Kinase Antibody #9102 (lower).

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic acetylated lysine-containing peptide. Antibodies are purified by protein A and peptide affinity chromatography.



Immunohistochemical staining of a paraffin-embedded human breast tumor section, showing nuclear and cytoplasmic localization of proteins with acetylated lysine residues using Acetylated-Lysine Antibody.

License/Use Restrictions: *Use of CST Motif Antibodies within certain methods (e.g., U.S. Patent No.'s 7,198,896 & 7,300,753) may require a license from CST. For information regarding academic licensing terms please have your technology transfer office contact CST Legal Department at CST_ip@cellsignal.com. For information regarding commercial licensing terms please contact CST Pharma Services Department at ptmscan@cellsignal.com.*

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 antibody.*

***Species cross-reactivity is determined by western blot.**

****Anti-rabbit secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

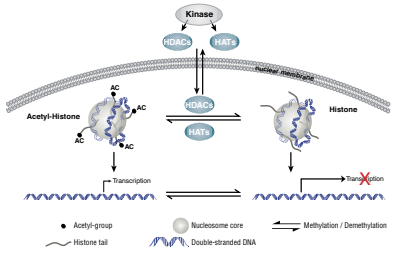
Western Blotting	1:1000
Immunoprecipitation	1:100
Immunohistochemistry (Paraffin)	1:800†
Unmasking buffer:	Citrate
Antibody diluent:	SignalStain® Antibody Diluent #8112
Detection reagent:	SignalStain® Boost (HRP, Rabbit) #8114

†*Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.*

Immunofluorescence (IF-IC)	1:100
ELISA-Peptide	1:1000
Chromatin IP	1:50

For product specific protocols please see the web page for this product at www.cellsignal.com.

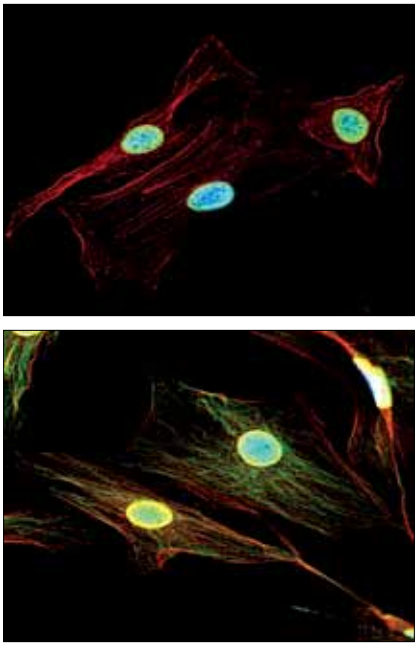
Please visit www.cellsignal.com for a complete listing of recommended complementary products.



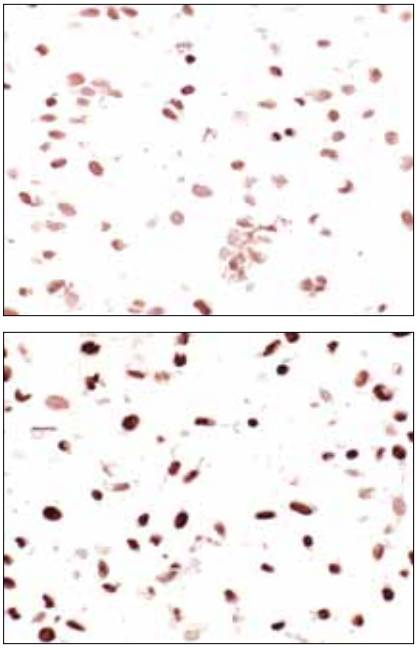
IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

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

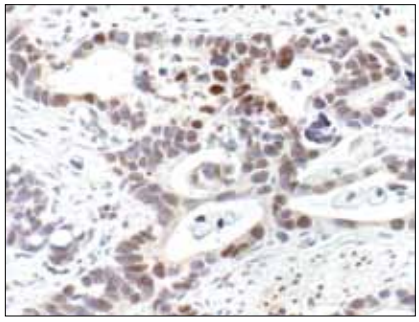
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed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



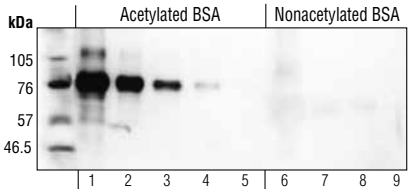
Confocal immunofluorescent analysis of NIH/3T3 cells, untreated (upper) or SAHA-treated (lower), labeled with Acetylated-Lysine Antibody (green). Actin filaments have been labeled with Alexa Fluor® 555 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).



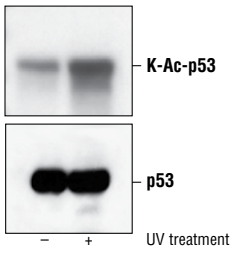
Immunohistochemical analysis of paraffin-embedded NIH/3T3 untreated (upper) or TSA-treated (lower) using Acetylated-Lysine Antibody.



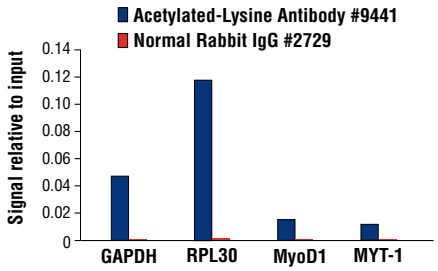
Immunoshitochemical analysis of paraffin-embedded human colon carcinoma using Acetylated-Lysine Antibody.



Specificity and sensitivity of Acetylated-Lysine Antibody assayed on acetylated BSA (4; 1; 0.2; 0.04 or 0.008 ng in lanes 1-5) or nonacetylated BSA (25,000; 5,000; 1,000 or 200 ng in lanes 6-9).



Western blot analysis of immunoprecipitated p53 showing an increase in p53 acetylation using Acetylated-Lysine Antibody (upper) or p53 antibody (lower). p53 was immunoprecipitated from lysates from 293 cells, untreated or UV-treated, using p53 Antibody #9282.



Chromatin immunoprecipitations were performed with cross-linked chromatin from 4 x 10⁶ HeLa cells and either 10 µl of Acetylated-Lysine Antibody or 2 µl of Normal Rabbit IgG #2729, using SimpleChIP® Enzymatic Chromatin IP Kit (Agarose Beads) #9002. The enriched DNA was quantified by real-time PCR, using SimpleChIP® Human GAPDH Exon 1 Primers #5516, SimpleChIP® Human RPL30 Exon 3 Primers #7014, SimpleChIP® Human MyoD1 Exon 1 Primers #4490, and SimpleChIP® Human MYT-1 Exon 1 Primers #4493. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

Background References:

(1) Hassig, C.A. and Schreiber, S.L. (1997) *Curr Opin Chem Biol* 1, 300-8.

(2) Allfrey, V.G. et al. (1964) *Proc Natl Acad Sci USA* 51, 786-94.

(3) Liu, L. et al. (1999) *Mol Cell Biol* 19, 1202-9.

(4) Boyes, J. et al. (1998) *Nature* 396, 594-8.

(5) Polevoda, B. and Sherman, F. (2002) *Genome Biol* 3, reviews0006.

(6) Yoshida, M. et al. (2003) *Prog Cell Cycle Res* 5, 269-78.

(7) Kim, S.C. et al. (2006) *Mol Cell* 23, 607-18.

(8) Choudhary, C. et al. (2009) *Science* 325, 834-40.

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(10) Vigushin, D.M. and Coombes, R.C. (2004) *Curr Cancer Drug Targets* 4, 205-18.