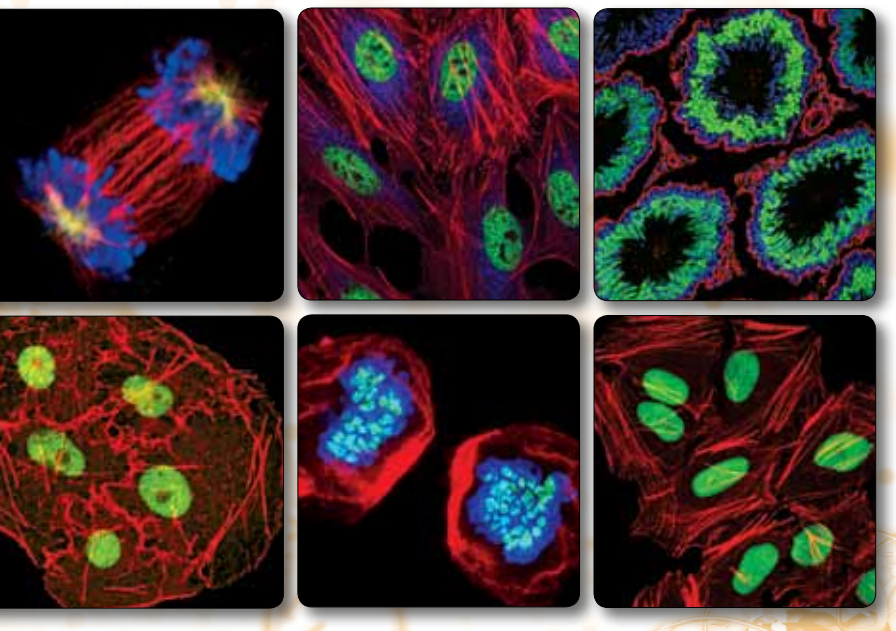
UNPARALLELED PRODUCT QUALITY, VALIDATION, AND TECHNICAL SUPPORT



Antibodies and Kits
for the Study of

# Chromatin and Epigenetic Regulation

Cell Signaling
TECHNOLOGY®

# XP™ Monoclonal Antibodies
## *for Chromatin and Epigenetic Regulation*

XP™ monoclonal antibodies are a line of high quality rabbit monoclonal antibodies exclusively available from Cell Signaling Technology (CST). Any product labeled with XP has been carefully selected based on superior performance in all approved applications.

XP monoclonal antibodies are generated using XMT™, a proprietary monoclonal technology developed at CST. The technology provides access to a broad range of antibody-producing B cells unattainable with traditional monoclonal technologies, allowing more comprehensive screening and the identification of XP monoclonal antibodies with:

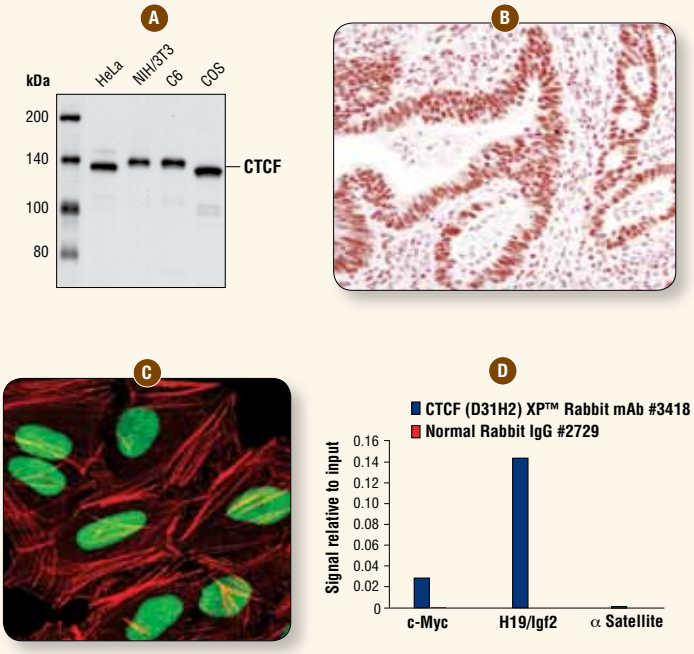
### eXceptional specificity
As with all CST™ antibodies, the antibody is specific to your target of interest, saving you valuable time and resources.

### +eXceptional sensitivity
The antibody will provide a stronger signal for your target protein in cells and tissues, allowing you to monitor expression of low levels of endogenous proteins, saving you valuable materials.

### +eXceptional stability and reproducibility
XMT combined with our stringent quality control ensures maximum lot-to-lot consistency and the most reproducible results.

### =eXceptional Performance™
XMT coupled with our extensive antibody validation and stringent quality control delivers XP monoclonal antibodies with eXceptional Performance in the widest range of applications.

**CTCF (D31H2) XP™ Rabbit mAb #3418 is an example of an antibody with superior performance in a wide range of tested applications.**



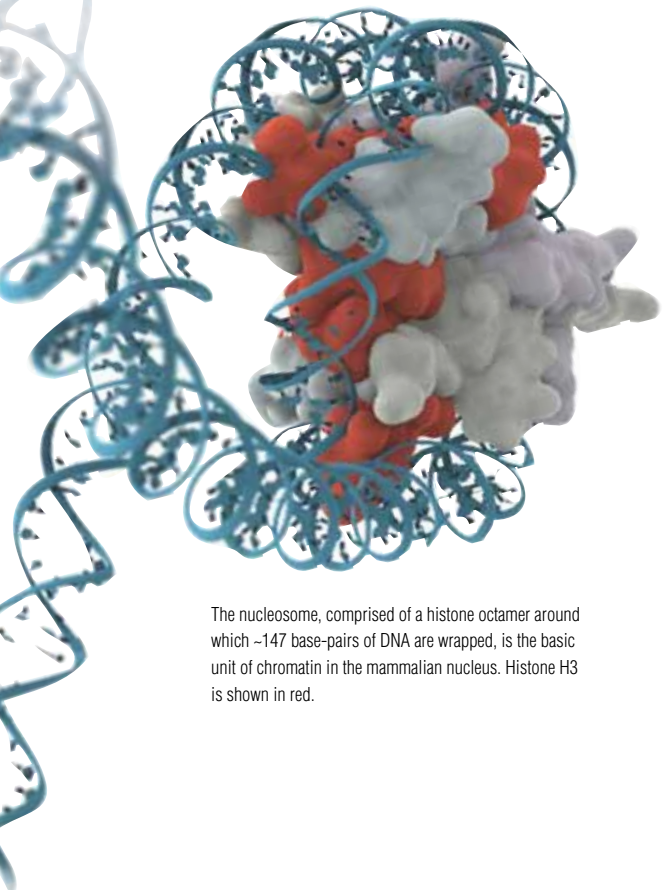

***CTCF (D31H2) XP™ Rabbit mAb #3418:*** ***(A)*** *WB analysis of extracts from various cell lines using #3418.* ***(B)*** *IHC analysis of paraffin-embedded human colon carcinoma using #3418.* ***(C)*** *Confocal IF analysis of HCT-116 cells using #3418 (green). Actin filaments were labeled with DY-554 phalloidin (red).* ***(D)*** *ChIP assays were performed with cross-linked chromatin from 4 x $10^6$ HeLa cells, with either 10 µl of #3418 or 2 µl of Normal Rabbit IgG #2729, using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using human c-Myc promoter primers, SimpleChIP® Human H19/Igf2 ICR Primers #5172, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin (equivalent to one).*

Visit our website for more experimental details, additional information, and a complete list of available XP Monoclonal Antibodies.

### Antibodies and Kits for the Study of

# Chromatin and Epigenetic Regulation

Cell Signaling Technology provides the highest quality activation-state and total protein antibodies for the study of chromatin and epigenetic regulation. CST™ antibodies have been extensively validated by our in-house scientists in applications including chromatin immunoprecipitation (ChIP), immunofluorescence, immunohistochemistry, and flow cytometry. Moreover, technical support is provided by the same scientists who develop and produce the antibodies and know them best. CST phosphorylation-specific antibodies are the most highly cited and serve as core reagents in multiple drug discovery platforms. Comprehensive and up-to-date information can be found at **www.cellsignal.com.**



The nucleosome, comprised of a histone octamer around which ~147 base-pairs of DNA are wrapped, is the basic unit of chromatin in the mammalian nucleus. Histone H3 is shown in red.

## Table of Contents

# SimpleChIP® Enzymatic Chromatin IP Kits

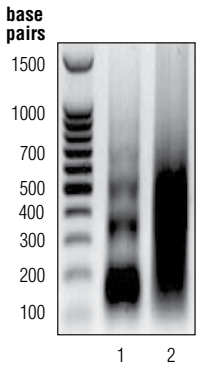
SimpleChIP® Enzymatic Chromatin IP Kits from Cell Signaling Technology (CST) are co-developed by CST and New England Biolabs and contain the highest quality research reagents. These kits are available with either Protein G agarose or Protein G magnetic beads and contain all buffers and reagents needed to perform up to 30 ChIP assays. The same reagents in these kits have been used for in-house ChIP validation at CST, which will simplify your optimization. The kits can be used with any ChIP-validated antibody to detect endogenous levels of protein-DNA interactions and histone modifications in mammalian cells. Protein G agarose and Protein G magnetic beads are also available as separate products.

Please visit www.cellsignal.com/technologies/chip.html for information on ChIP and CST™ SimpleChIP products.

## The SimpleChIP® Kit Advantage

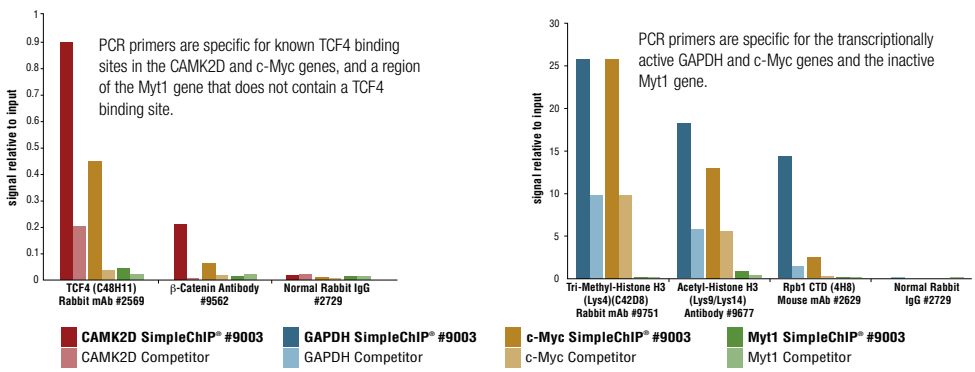
**Enzyme-based Chromatin Digestion vs. Sonication-based Chromatin Fragmentation:** Prior to performing chromatin IP, it is important to process chromatin to the appropriate DNA fragment size. CST SimpleChIP Enzymatic Chromatin IP Kits use micrococcal nuclease digestion to obtain chromatin fragments, whereas many competitor kits use a sonication-based method.

| | SimpleChIP® from Cell Signaling Technology | Competitor Kits |
|---|---|---|
| Fragmentation Method | *Enzymatic* | *Sonication* |
| Chromatin Quality | *High (mild sample treatment preserves chromatin integrity and antibody epitopes)* | *Low (rigorous sample treatment disrupts chromatin integrity and antibody epitopes)* |
| IP Efficiency | *High* | *Low* |
| Detection | *Higher sensitivity (especially crucial for transcription factors and cofactors)* | *Lower sensitivity (significantly decreased signal for less abundant chromatin binding proteins)* |




***Enzyme-based and sonication-based ChIP kits produce chromatin fragments of a similar size, but differing chromatin integrity.*** *Chromatin was prepared from 4 x $10^7$ HCT 116 human colorectal carcinoma cells according to the protocols included with the SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003 and from a competitor's sonication-based ChIP kit. DNA was purified from each chromatin sample and DNA fragment size was determined by electrophoresis on a 1% agarose gel. Both enzymatic digestion with the SimpleChIP® Kit (lane 1) and sonication with the competitor's kit (lane 2) produced chromatin fragments ranging from 150 to 700 bp, corresponding to one to five nucleosomes in length.*

### Enzyme-based Chromatin Digestion vs. Sonication-based Chromatin Fragmentation




***SimpleChIP® digested chromatin is more conducive to immunoprecipitation than sonicated chromatin.*** *ChIP assays were performed with 10 µg of cross-linked HCT 116 chromatin and the indicated antibodies, using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003 and a competitor's sonication-based ChIP Kit. The enriched DNA was quantified by qPCR. The amount of immunoprecipitated DNA in each sample is presented as a percent of the total input chromatin. For every target tested, enzyme-digested chromatin showed better enrichment of target DNA loci than did sonicated chromatin.*

## SimpleChIP® Related Products

| | | Reactivity |
|---|---|---|
| #9002 | SimpleChIP® Enzymatic Chromatin IP Kit (Agarose Beads) | H, M, R, Mk |
| #9003 | SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) | H, M, R, Mk |
| #7017 | 6-Tube Magnetic Separation Rack | |
| #9007 | ChIP-Grade Protein G Agarose Beads | |
| #9006 | ChIP-Grade Protein G Magnetic Beads | |
| #2729 | Normal Rabbit IgG | |
| #5415 | Mouse (G3A1) mAb IgG1 Isotype Control | |

## SimpleChIP® Control PCR Primers

SimpleChIP Control PCR Primers featured throughout this brochure are a mix of forward and reverse primers that can be used to amplify DNA isolated using ChIP. These primers amplify positive control DNA sequences that contain known binding sites of the target protein detected by the antibody employed in the ChIP assay, and can also be used as a negative control to demonstrate antibody sensitivity.

- Primers are designed, tested, and optimized in-house in conjunction with our ChIP-validated antibodies and SimpleChIP Kits, saving time and reagents.
- Primers are optimized for use in real-time PCR with SYBR® Green dye, which simplifies quantification of DNA enrichment.
- Technical Support is provided by the scientists who designed and use these products, and know them best, assuring a fast and accurate response.



REACTIVITY KEY:
**H** human / **M** mouse / **R** rat / **Hm** hamster / **Mk** monkey / **C** chicken / **Mi** mink / **Dm** D. melanogaster / **X** Xenopus / **Z** zebra fish / **B** bovine / **Dg** dog / **Pg** pig / **Sc** S. cerevisiae / **All** all species expected / **( )** 100% sequence homology

# SimpleChIP® Enzymatic Chromatin IP Kits

**30 assays**

**#9002 SimpleChIP® Enzymatic Chromatin IP Kit (Agarose Beads)**

Glycine Solution (10X)
Buffer A (4X)
Buffer B (4X)
ChIP Buffer (10X)
ChIP Elution Buffer (2X)
5 M NaCl
0.5 M EDTA
DNA Binding Buffer
DNA Wash Buffer
DNA Elution Buffer
RNAse A (10 mg/ml)
Micrococcal Nuclease
ChIP-Grade Protein G Agarose Beads (blocked with BSA and sonicated salmon sperm DNA) #9007
DNA Purification Columns
Protease Inhibitor Cocktail (200X)
Proteinase K
SimpleChIP® Human RPL30 Exon 3 Primers #7014
SimpleChIP® Mouse RPL30 Intron 2 Primers #7015
Histone H3 (D2B12) XP™ Rabbit mAb (ChIP Formulated) #4620
Normal Rabbit IgG #2729
1M DTT

**#9003 SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads)**

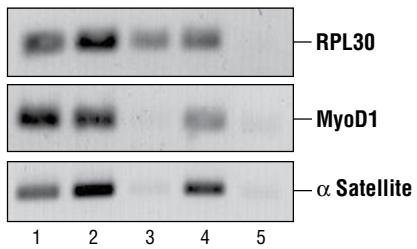
This kit contains the same components as #9002 except #9003 contains ChIP-Grade Protein G Magnetic Beads #9006 (blocked with BSA).

**Selected Application References:**

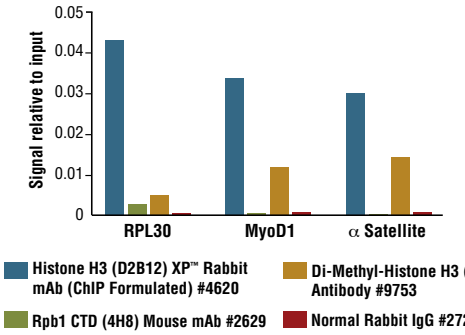
**SimpleChIP® Enzymatic Chromatin IP Kit (Agarose Beads) #9002:**
Czymai, T. et al. (2010) *J. Biol. Chem.* 285, 10163-10178. (ChIP)
Ghosh, R. et al. (2010) *PLoS One* 5, e9575. (ChIP)

**SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003:**
Bommer, G.T. et al. (2010) *J. Biol. Chem.* 285, 1928-1938. (ChIP)
De Bruyne, E. et al. (2010) *Blood* 115, 2430-2440. (ChIP)
Onishi, Y. (2010) *Biosci. Rep.* 31, 57-62. (ChIP)

***SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003:***




*ChIP assays were performed using digested chromatin from HeLa cells and either Histone H3 (D2B12) XP™ Rabbit mAb (ChIP Formulated) #4620 (lane 2), Rpb1 CTD (4H8) Mouse mAb #2629 (lane 3), Di-Methyl Histone H3 (Lys9) Antibody #9753 (lane 4), or Normal Rabbit IgG #2729 (lane 5). Purified DNA was analyzed by standard PCR methods using SimpleChIP® Human RPL30 Exon 3 Primers #7014, SimpleChIP® Human MyoD1 Exon 1 Primers #4490, and SimpleChIP® Human α Satellite Repeat Primers #4486. PCR products were observed for each primer set in the input sample (lane 1) and various ChIP samples, but not in the Normal Rabbit IgG ChIP sample (lane 5).*




*ChIP assays were performed using digested chromatin from HeLa cells and the indicated ChIP-validated antibodies. Purified DNA was analyzed by quantitative real-time PCR using SimpleChIP® Human RPL30 Exon 3 Primers #7014 (control primer set), SimpleChIP® Human MyoD1 Exon 1 Primers #4490, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin (equivalent to one).*

# SimpleChIP® Assay Kits

New SimpleChIP Assay Kits combine several ChIP-validated antibodies with control PCR primer mixes that serve as markers for pluripotency or epigenetic status. Rigorous in-house quality control and testing ensure the antibodies included in the kits meet the highest standards for quality, validation, and lot-to-lot consistency. The kits provide all reagents necessary to perform 10 ChIP assays and subsequent real-time PCR reactions. Pre-selected positive and negative primer sets are included in each kit, providing proven and appropriate controls for customer experiments.

**10 assays**

**#8980 SimpleChIP® Stem Cell Master Regulator Assay Kit**

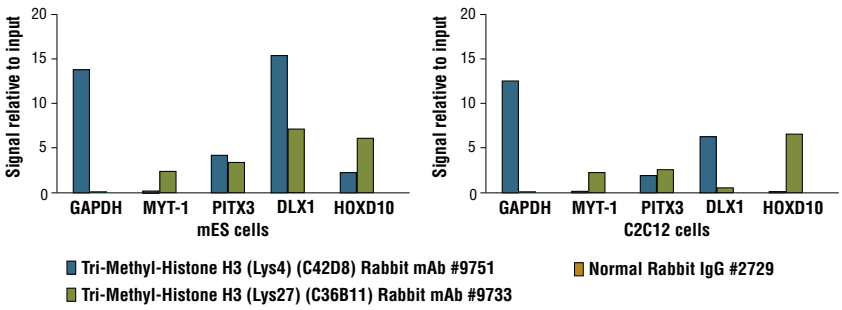
Nanog (D73G4) XP™ Rabbit mAb (ChIP Formulated) #5232
Oct-4A (C30A3C1) Rabbit mAb (ChIP Formulated) #5677
Sox2 (D6D9) XP™ Rabbit mAb (ChIP Formulated) #5024
SimpleChIP® Human Oct-4 Promoter Primers #4641
SimpleChIP® Human α Satellite Repeat Primers #4486

**#8982 SimpleChIP® Human Bivalent Promoter Assay Kit**

Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb #9751
Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb #9733
SimpleChIP® Human GAPDH Exon 1 Primers #5516
SimpleChIP® Human MYT-1 Exon 1 Primers #4493
SimpleChIP® Human GATA6 Promoter Primers #5550

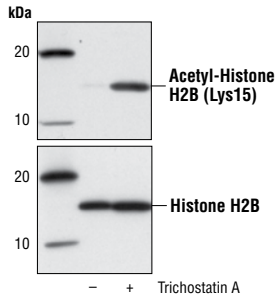
**#8981 SimpleChIP® Mouse Bivalent Promoter Assay Kit**

Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb #9751
Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb #9733
SimpleChIP® Mouse GAPDH Intron 2 Primers #8986
SimpleChIP® Mouse MYT-1 Promoter Primers #8985
SimpleChIP® Mouse PITX3 Intron 1 Primers #8984

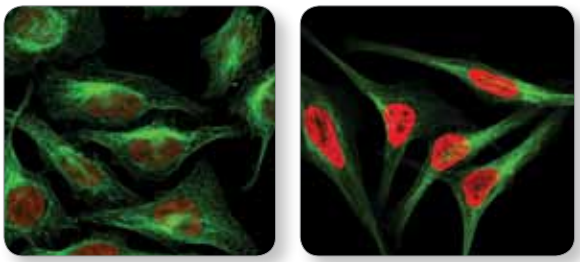



***SimpleChIP® Mouse Bivalent Promoter Assay Kit #8981:*** *ChIP assays were performed with cross-linked chromatin from 4 x $10^6$ mouse embryonic stem (mES) cells (left panel) or C2C12 cells (right panel) and Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb #9751, Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb #9733, or 2 µl of Normal Rabbit IgG #2729, using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using SimpleChIP® Mouse GAPDH Intron 2 Primers #8986, SimpleChIP® Mouse MYT-1 Promoter Primers #8985, SimpleChIP® Mouse PITX3 Intron 1 Primers #8984, mouse DLX1 promoter primers, and mouse HOXD10 intron 1 primers. The amount of immunoprecipitated DNA in each sample is normalized for enrichment of total histone H3 and represented as signal relative to the total amount of input chromatin (equivalent to one). Note that the PITX3, DLX1, and HOXD10 promoters are all bivalent in stem cells, while only PITX3 remains bivalent in the differentiated cell line C2C12.*

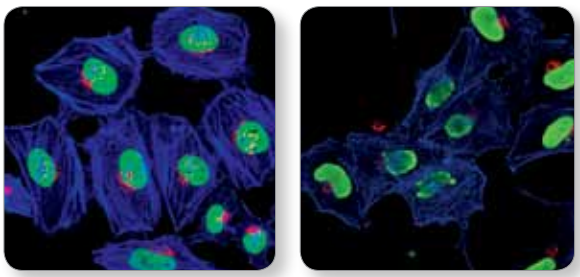
# Histones and Histone Modifications



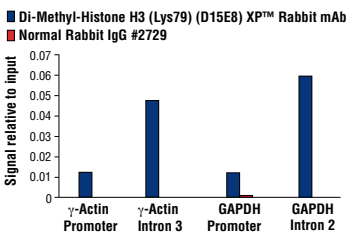

◀ ***Acetyl-Histone H2B (Lys15) Antibody #5435:*** *WB analysis of extracts from HeLa cells, untreated or treated with Trichostatin A (TSA) #9950 (1 µM for 18 h), using #5435 (upper) and Histone H2B (53H3) Mouse mAb #2934 (lower).*



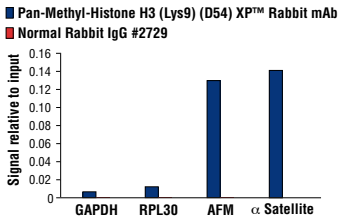
***Acetyl-Histone H3 (Lys9) (C5B11) Rabbit mAb (Alexa Fluor® 555 Conjugate) #5489:*** *Confocal IF analysis of HeLa cells, untreated (left) or treated with Trichostatin A (TSA) #9950 (right), using #5489 (red) and α-Tubulin (DM1A) Mouse mAb #3873 (green).*



***Acetyl-Histone H4 (Lys5) Antibody #9672:*** *Confocal IF analysis of HeLa cells, untreated (left) or treated with Trichostatin A (TSA) #9950 (right), using #9672 (green) and Golgin-97 Antibody (red). Actin filaments were labeled with a dye-conjugated phalloidin (blue pseudocolor).*




***Di-Methyl-Histone H3 (Lys79) (D15E8) XP™ Rabbit mAb #5427:*** *ChIP assays were performed with cross-linked chromatin from 4 x $10^6$ HeLa cells and either 10 µl of #5427 or 2 µl of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human γ-Actin Promoter Primers #5037, SimpleChIP® Human γ-Actin Intron 3 Primers #5047, SimpleChIP® Human GAPDH Promoter Primers #4471, and SimpleChIP® Human GAPDH Intron 2 Primers #4478. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin (equivalent to one).*




***Pan-Methyl-Histone H3 (Lys9) (D54) XP™ Rabbit mAb #4473:*** *ChIP assays were performed with cross-linked chromatin from 4 x $10^6$ HeLa cells and either 20 µl of #4473 or 2 µl of Normal Rabbit IgG #2729, using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. 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 AFM Intron 1 Primers #5098, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin (equivalent to one).*

## Acetylation

| | | | Application | Reactivity |
|---|---|---|---|---|
| | NEW | **#9814 Acetylated-Lysine (Ac-K2-100) Rabbit mAb** | W, IP, ChIP, E-P | All |
| | | **#9681 Acetylated-Lysine Mouse mAb (Ac-K-103)** | W, E-P | All |
| | | **#9441 Acetylated-Lysine Antibody** | W, IP, IHC-P, IF-IC, ChIP, E-P | All |
| **H2A** | | **#2576 Acetyl-Histone H2A (Lys5) Antibody** | W, IP, IHC-P | H, M, R, Mk |
| **H2B** | | **#2574 Acetyl-Histone H2B (Lys5) Antibody** | W, IP, IHC-P | H, M, R, Mk |
| | NEW | **#5410 Acetyl-Histone H2B (Lys12) Antibody** | W, IP, IF-IC | H, M, R, Mk |
| | NEW | **#5435 Acetyl-Histone H2B (Lys15) Antibody** *featured* | W, IF-IC | H, M, R, Mk, (B, Pg) |
| | | **#2571 Acetyl-Histone H2B (Lys20) Antibody** | W, IP, IHC-P, IF-IC, IC | H, M, R |
| **H3** | | **#9649 Acetyl-Histone H3 (Lys9) (C5B11) Rabbit mAb** | W, IHC-P, IF-IC, F, ChIP | H, M, R, Mk, Z |
| | NEW | **#9683 Acetyl-Histone H3 (Lys9) (C5B11) Rabbit mAb (Alexa Fluor® 488 Conjugate)** | IF-IC, F | H, M, Z, (R, Mk) |
| | NEW | **#5489 Acetyl-Histone H3 (Lys9) (C5B11) Rabbit mAb (Alexa Fluor® 555 Conjugate)** *featured* | IF-IC | H, M, Z, (R, Mk) |
| | NEW | **#4484 Acetyl-Histone H3 (Lys9) (C5B11) Rabbit mAb (Alexa Fluor® 647 Conjugate)** | IF-IC, F | H, M, Z, (R, Mk) |
| | | **#9711 Acetyl- and Phospho-Histone H3 (Lys9/Ser10) Antibody** | W, IHC-P, IF-P | H, M, R |
| | | **#9671 Acetyl-Histone H3 (Lys9) Antibody** | W, IP, IHC-P, ChIP | H, M, R, Mk, Dm, Sc |
| | | **#9677 Acetyl-Histone H3 (Lys9/Lys14) Antibody** | W, IP, ChIP | H, M, R, Mk, (Z) |
| | NEW | **#4318 Acetyl-Histone H3 (Lys14) Antibody** | W, IF-IC | H, M, R, Mk, (Dm) |
| | NEW | **#5275 Acetyl-Histone H3 (Lys14) Antibody (ChIP Formulated)** *featured* | ChIP | H, (M, R, Mk) |
| | | **#9675 Acetyl-Histone H3 (Lys18) Antibody** | W, IHC-P, ChIP | H, M, R |
| | | **#9674 Acetyl-Histone H3 (Lys23) Antibody** | W, IHC-P | H, M, R |
| | NEW | **#4353 Acetyl-Histone H3 (Lys27) Antibody** | W, IP, ChIP | H, M, R, Mk, (Hm, C, Dm, X, Z, B) |
| | NEW | **#4243 Acetyl-Histone H3 (Lys56) Antibody** | W, IP, IF-IC | H, M, R, Mk |
| **H4** | NEW | **#9672 Acetyl-Histone H4 (Lys5) Antibody** *featured* | W, IP, IHC-P, IF-IC, ChIP | H, M, R, Mk, (C, Dm, X, Z, B, Pg) |
| | | **#2594 Acetyl-Histone H4 (Lys8) Antibody** | W, IF-IC, F, ChIP | H, M, R, Mk |
| | | **#2591 Acetyl-Histone H4 (Lys12) Antibody** | W, IHC-P, IF-IC, F | H, M, R, Mk |

### Selected Application References:

**Acetylated-Lysine Mouse mAb (Ac-K-103) #9681:**


VanDemark, A.P. et al. (2007) *Mol. Cell* 27, 817-828. (W)

Zhao, L.J. et al. (2006) *J. Biol. Chem.* 281, 36613-36623. (W)


**Acetylated-Lysine Antibody #9441:**

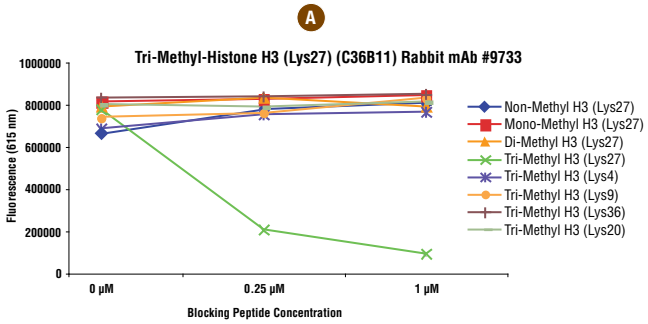

Werner, H.B. et al. (2007) *J. Neurosci.* 27, 7717-7730. (W)

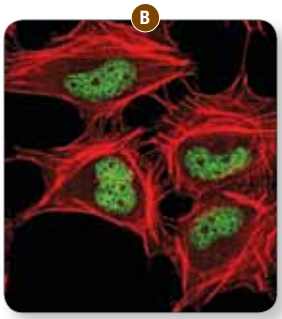

**Acetyl-Histone H3 (Lys9) (C5B11) Rabbit mAb #9649:**


Kong, D.K. et al. (2010) *Mol. Biol. Cell* 21, 1335-1349. (ChIP)




APPLICATIONS KEY:
**W** Western / **IP** Immunoprecipitation / **IHC** Immunohistochemistry / **IF** Immunofluorescence / **F** Flow Cytometry / **ChIP** Chromatin Immunoprecipitation / (**-IC** Immunocytochemistry, **-P** Paraffin, **-F** Frozen) / **E-P** Peptide ELISA

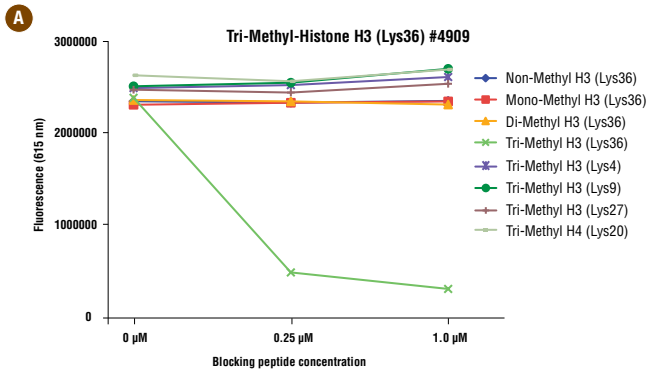


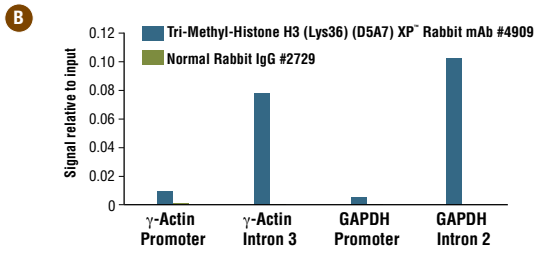




***Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb #9733:*** *(A) Specificity of the antibody was determined by peptide ELISA. The graph depicts the binding of the antibody to pre-coated tri-methyl histone H3 (Lys27) peptide in the presence of increasing concentrations of various competitor peptides. As shown, only the tri-methyl histone H3 (Lys27) peptide (green) competed away binding of the antibody.* ***(B)*** *Confocal IF analysis of HeLa cells using #9733 (green). Actin filaments were labeled with DY-554 phalloidin (red).*

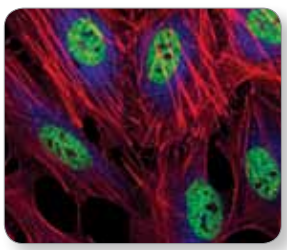
## Methylation

| | | | Application | Reactivity |
|---|---|---|---|---|
| **H3** | **#9707** | **Methyl-Histone H3 (Arg2) Antibody** | W | H |
| NEW | **#5326** | **Mono-Methyl-Histone H3 (Lys4) (D1A9) XP™ Rabbit mAb** *featured* | W, IF-IC, ChIP | H, M, R, Mk |
| | **#9723** | **Mono-Methyl-Histone H3 (Lys4) Antibody** | W, IP, IF-IC | H, M, R, Mk, (X, Z) |
| | **#9725** | **Di-Methyl-Histone H3 (Lys4) (C64G9) Rabbit mAb** | W, IP, IHC-P, IF-IC, ChIP | H, M, R, Mk |
| | **#9726** | **Di-Methyl-Histone H3 (Lys4) Antibody** | W, IP, IHC-P, IF-IC, ChIP | H, M, R, Mk, (X, Z) |
| | **#9751** | **Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb** | W, IHC-P, IF-IC, ChIP | H, M, R, Mk, Dm, Sc, (X, Z) |
| | **#9727** | **Tri-Methyl-Histone H3 (Lys4) Antibody** | W, IP, IHC-P, IF-IC, ChIP | H, M, R, Mk, (X, Z) |
| NEW | **#4658** | **Di-Methyl-Histone H3 (Lys9) (D85B4) XP™ Rabbit mAb** | W, IP, IF-IC, ChIP | H, M, R, Mk, (Dm, X, Z, B, Pg, Sc) |
| | **#9753** | **Di-Methyl-Histone H3 (Lys9) Antibody** | W, IP, IHC-P, IF-IC, ChIP | H, M, R, Mk, Dm, Sc |
| NEW | **#5327** | **Di/Tri-Methyl-Histone H3 (Lys9) (6F12) Mouse mAb** | W, IP, IF-IC, ChIP | H, M, R, Mk |
| | **#9754** | **Tri-Methyl-Histone H3 (Lys9) Antibody** | W, IF-IC, ChIP | H, M, R, Mk, (Dm, Pg) |
| NEW | **#4473** | **Pan-Methyl-Histone H3 (Lys9) (D54) XP™ Rabbit mAb** *featured* | W, IP, IF-IC, ChIP | H, M, R, Mk, (C, Dm, X, Z, B, Pg, Sc) |
| | **#4069** | **Pan-Methyl-Histone H3 (Lys9) Antibody** | W, IP, IF-IC, ChIP | H, M, R, Mk, Z |
| | **#9728** | **Di-Methyl-Histone H3 (Lys27) (D18C8) XP™ Rabbit mAb** | W, IF-IC, ChIP | H, M, R, Mk |
| | **#9755** | **Di-Methyl-Histone H3 (Lys27) Antibody** | W, IP, IF-IC | H, M, R, Mk |
| | **#9733** | **Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb** *featured* | W, IP, IHC-P, IF-IC, ChIP | H, M, R, Mk, (X, Z) |
| | **#9756** | **Tri-Methyl-Histone H3 (Lys27) Antibody** | W, IP, IHC-P, IF-IC, ChIP | H, M, R, Mk, (X) |
| | **#2901** | **Di-Methyl-Histone H3 (Lys36) (C75H12) Rabbit mAb** | W, IHC-P, IF-IC | H, M, R, Mk |
| | **#9758** | **Di-Methyl-Histone H3 (Lys36) Antibody** | W, IP, IF-IC | H, M, R, Mk |
| NEW | **#4909** | **Tri-Methyl-Histone H3 (Lys36) (D5A7) XP™ Rabbit mAb** *featured* | W, ChIP | H, M, R, Mk, (Hm, C, Dm, X, Z, B) |
| | **#9763** | **Tri-Methyl-Histone H3 (Lys36) Antibody** | W, IHC-P, IF-IC | H, M, R, Mk |
| NEW | **#5427** | **Di-Methyl-Histone H3 (Lys79) (D15E8) XP™ Rabbit mAb** *featured* | W, ChIP | H, M, R, Mk |
| | **#9757** | **Di-Methyl-Histone H3 (Lys79) Antibody** | W, IP | H, M, R, Mk |
| **H4** | **#9724** | **Mono-Methyl-Histone H4 (Lys20) Antibody** | W | H, M, R, Mk, (Dm, X, Z, B, Pg) |
| | **#9759** | **Di-Methyl-Histone H4 (Lys20) Antibody** | W | H, M, R, Mk, (Dm, Z, B, Pg,) |
| NEW | **#5737** | **Tri-Methyl-Histone H4 (Lys20) Rabbit mAb** | W, IP, ChIP | H, M, R, Mk |

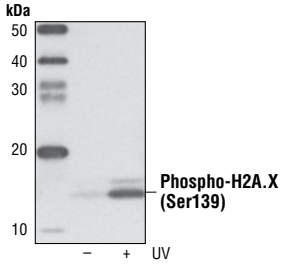


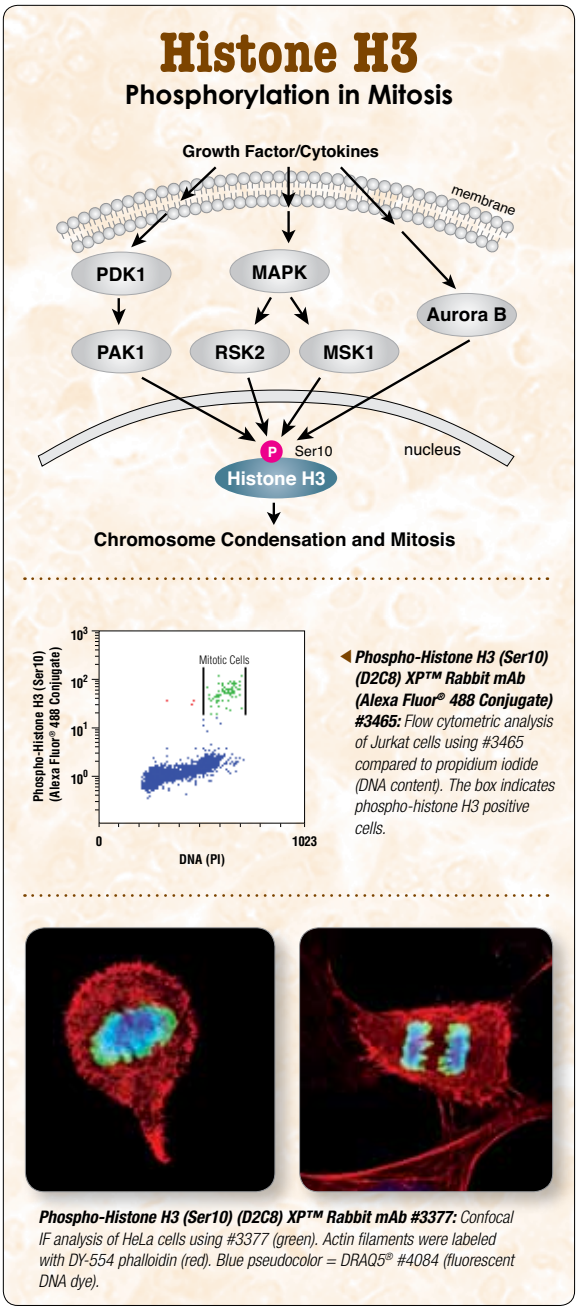

***Tri-Methyl-Histone H3 (Lys36) (D5A7) XP™ Rabbit mAb #4909:*** *Specificity of the antibody was determined by peptide ELISA. The graph* ***(A)*** *depicts the binding of the antibody to pre-coated tri-methyl histone H3 (Lys36) peptide in the presence of increasing concentrations of various competitor peptides. As shown, only the tri-methyl histone H3 (Lys36) peptide (green) competed away binding of the antibody.* ***(B)*** *ChIP assays were performed with cross-linked chromatin from 4 x $10^6$ HeLa cells and either 10 µl of #4909 or 2 µl of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human γ-Actin Promoter Primers #5037, SimpleChIP® Human γ-Actin Intron 3 Primers #5047, SimpleChIP® Human GAPDH Promoter Primers #4471, and SimpleChIP® Human GAPDH Intron 2 Primers #4478. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin (equivalent to one).*



***Mono-Methyl-Histone H3 (Lys4) (D1A9) XP™ Rabbit mAb #5326:*** *Confocal IF analysis of HeLa cells using #5326 (green) and MEK1/2 (L38C12) Mouse mAb #4694 (blue). Actin filaments were labeled with DY-554 phalloidin (red).*




***Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb (Biotinylated) #4309:*** *WB analysis of extracts from HT-29 cells, untreated or UV-treated, using #4309 and detected with Streptavidin-HRP #3999.*







***Phospho-Histone H3 (Ser10) (D2C8) XP™ Rabbit mAb (Alexa Fluor® 488 Conjugate) #3465:*** *Flow cytometric analysis of Jurkat cells using #3465 compared to propidium iodide (DNA content). The box indicates phospho-histone H3 positive cells.*



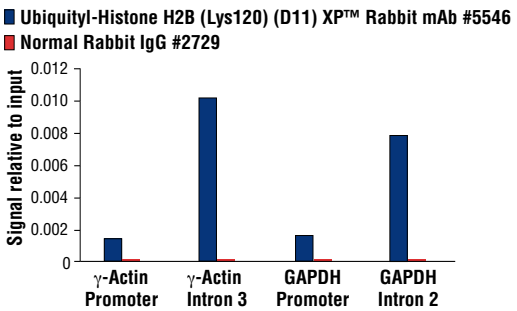
***Phospho-Histone H3 (Ser10) (D2C8) XP™ Rabbit mAb #3377:*** *Confocal IF analysis of HeLa cells using #3377 (green). Actin filaments were labeled with DY-554 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).*

## Phosphorylation

| | | | Application | Reactivity |
|---|---|---|---|---|
| **H2A.X** | #9718 | **Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb** | W, IHC-P, IF-IC, F | H, M, R, Mk |
| | #9719 | **Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb (Alexa Fluor® 488 Conjugate)** | IF-IC, F | H, M |
| | #9720 | **Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb (Alexa Fluor® 647 Conjugate)** | IF-IC, F | H, M |
| | NEW #4309 | **Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb (Biotinylated)** *featured* | W | H, M, R, Mk |
| | #2577 | **Phospho-Histone H2A.X (Ser139) Antibody** | W, IHC-P, IF-IC, F | H, M, R |
| | NEW #5438 | **Phospho-Histone H2A.X (Ser139/Tyr142) Antibody** | W, IP, IF, F | H, M, R, Mk |
| **H3** | #9714 | **Phospho-Histone H3 (Thr3) Antibody** | W, IHC-P | H, M, R |
| | #3377 | **Phospho-Histone H3 (Ser10) (D2C8) XP™ Rabbit mAb** *featured* | W, IF-IC, F | H, M, R, Mk, Z |
| | #3465 | **Phospho-Histone H3 (Ser10) (D2C8) XP™ Rabbit mAb (Alexa Fluor® 488 Conjugate)** *featured* | IF-IC, F | H, M, R, Mk |
| | #3475 | **Phospho-Histone H3 (Ser10) (D2C8) XP™ Rabbit mAb (Alexa Fluor® 555 Conjugate)** | IF-IC | H, M, R, Mk |
| | #3458 | **Phospho-Histone H3 (Ser10) (D2C8) XP™ Rabbit mAb (Alexa Fluor® 647 Conjugate)** | IF-IC, F | H, M, R, Mk |
| | #3642 | **Phospho-Histone H3 (Ser10) (D2C8) XP™ Rabbit mAb (Biotinylated)** | W, IF-F, IF-IC, F | H, M, R, Mk |
| | #9706 | **Phospho-Histone H3 (Ser10) (6G3) Mouse mAb** | W, IF-F, IF-IC, F | H, M, R |
| | #9701 | **Phospho-Histone H3 (Ser10) Antibody** | W, IP, IHC-P, IHC-F, IF-IC, F | H, M, R, Mk, C, Dm, Z, Sc, (X) |
| | #9708 | **Phospho-Histone H3 (Ser10) Antibody (Alexa Fluor® 488 Conjugate)** | IF-IC, F | H, M, (X) |
| | #9716 | **Phospho-Histone H3 (Ser10) Antibody (Alexa Fluor® 647 Conjugate)** | IF-IC, F | H, M, R, Mk |
| | #9767 | **Phospho-Histone H3 (Thr11) (C2A6) Rabbit mAb** | W, IP, F | H, M, R, (X) |
| | #9764 | **Phospho-Histone H3 (Thr11) Antibody** | W, IP, IF-IC, F | H, M, R, (X) |
| | #9713 | **Phospho-Histone H3 (Ser28) Antibody** | W, IP, IF-F, IF-IC, F | H, M, Hm, Dm, (R, C, X, Z, B) |
| **CENP-A** | #2187 | **Phospho-CENP-A (Ser7) Antibody** *featured* | W, IP, IF-IC | H, (Mk) |

## Ubiquitylation

| | | | Application | Reactivity |
|---|---|---|---|---|
| **H2B** | NEW #5546 | **Ubiquityl-Histone H2B (Lys120) (D11) XP™ Rabbit mAb** *featured* | W, ChIP | H, M, R, Mk |




***Ubiquityl-Histone H2B (Lys120) (D11) XP™ Rabbit mAb #5546:*** *ChIP assays were performed with cross-linked chromatin from 4 x $10^6$ HeLa cells and either 10 µl of #5546 or 2 µl of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human γ-Actin Promoter Primers #5037, SimpleChIP® Human γ-Actin Intron 3 Primers #5047, SimpleChIP® Human GAPDH Promoter Primers #4471, and SimpleChIP® Human GAPDH Intron 2 Primers #4478. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin (equivalent to one).*

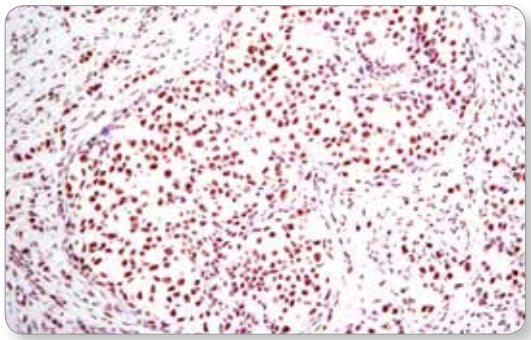


APPLICATIONS KEY:
**W** Western / **IP** Immunoprecipitation / **IHC** Immunohistochemistry / **IF** Immunofluorescence / **F** Flow Cytometry / **ChIP** Chromatin Immunoprecipitation / (**-IC** Immunocytochemistry, **-P** Paraffin, **-F** Frozen) / **E-P** Peptide ELISA

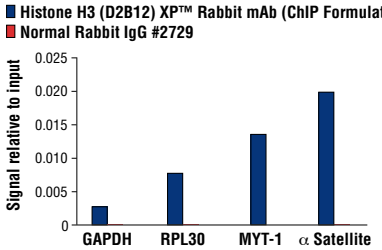
## Unmodified Histones and Variants

| | | | Application | Reactivity |
|---|---|---|---|---|
| **H2A** | | **#3636 Histone H2A (L88A6) Mouse mAb** | W, IHC-P | H, M, R, Mk, (C, Dm, Z, Pg) |
| | | **#2578 Histone H2A Antibody II** | W, IP, IHC-P | H, M, R, Mk, (X) |
| | | **#2595 Histone H2A.X Antibody** | W | H, Mk |
| | | **#2718 Histone H2A.Z Antibody** | W, IP, IF-IC | H, M, R, Mk, Z, (C, X, B) |
| **MACRO-H2A** | NEW | **#4160 MacroH2A1.1 Antibody** | W | H, M, R |
| | NEW | **#4827 MacroH2A1.2 Antibody** *featured* | W, IF-IC | H, M, R, Mk, (C, B) |
| **H2B** | | **#2722 Histone H2B Antibody** | W, IP | H, M, R, Mk |
| | | **#2934 Histone H2B (53H3) Mouse mAb** | W | H, M, R, Mk, Z, (X, B) |
| **H3** | NEW | **#4499 Histone H3 (D1H2) XP™ Rabbit mAb** *featured* | W, IHC-P, IF-IC | H, M, R, Mk, (Hm, C, Dm, X, Z, B) |
| | NEW | **#4620 Histone H3 (D2B12) XP™ Rabbit mAb (ChIP Formulated)** *featured* | ChIP | H, M, (R, Hm, Mk, C, Dm, X, Z, B) |
| | | **#9717 Histone H3 (3H1) Rabbit mAb** | W | H, M, R, Hm, Mk, Z, B, Pg |
| | NEW | **#5192 Histone H3 (3H1) Rabbit mAb (HRP Conjugate)** | W | H, M, R, Hm, Mk, Z, B, Pg |
| | | **#3638 Histone H3 (96C10) Mouse mAb** | W | H, M, R, Mk, (Dm, X) |
| | | **#3680 Histone H3 (96C10) Mouse mAb (IHC Formulated)** | IHC-P | H, (M, R, Mk, Dm, X) |
| | | **#9715 Histone H3 Antibody** | W, IHC-P, IF-IC | H, M, R, Mk, Z, B, Pg, (Dm) |
| | | **#2650 Histone H3 Antibody (ChIP Formulated)** | ChIP | H, M, (R, Mk, C, Dm, X, Z, B) |
| **H4** | | **#2935 Histone H4 (L64C1) Mouse mAb** | W, IHC-P | H, M, R, Mk, Z, (Dm, X, Z, B) |
| | | **#2960 Histone H4 (L64C1) Mouse mAb (ChIP Formulated)** | ChIP | H, (M, R, Mk, Dm, X, Z, B) |
| | | **#2592 Histone H4 Antibody** | W, IP, IHC-P | H, M, R, Mk, Dm, Z, Sc |
| **CENP-A** | | **#2048 CENP-A (C51A7) Rabbit mAb (Mouse Specific; IF Preferred)** | W, IF-IC | M |
| | | **#2047 CENP-A (C5H3) Rabbit mAb (Mouse Specific)** | W, IP | M |
| | | **#2186 CENP-A Antibody** | W, IF-IC | H |

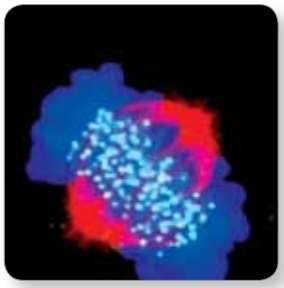
Cell Signaling Technology offers the highest quality research products, rigorous validation, and technical support provided by the same scientists who produce and validate the products and know them best.



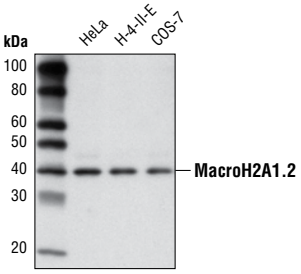
***Histone H3 (D1H2) XP™ Rabbit mAb #4499:*** *IHC analysis of paraffin-embedded human breast carcinoma using #4499.*




***Histone H3 (D2B12) XP™ Rabbit mAb (ChIP Formulated) #4620:*** *ChIP assays were performed with cross-linked chromatin from 4 x $10^6$ HeLa cells and either 10 µl of #4620 or 2 µl of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. 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 MYT-1 Exon 1 Primers #4493, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin (equivalent to one).*

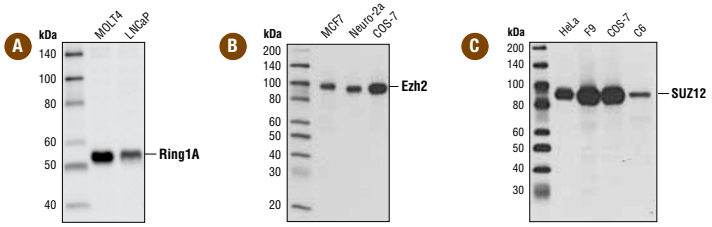


***Phospho-CENP-A (Ser7) Antibody #2187:*** *Confocal IF analysis of a mitotic HeLa cell using #2187 (green) and β-Tubulin (9F3) Rabbit mAb (Alexa Fluor® 555 Conjugate) #2116 (red). Phospho-CENP-A signal is localized to bright spots in the metaphase plate. Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).*




***MacroH2A1.2 Antibody #4827:*** *WB analysis of extracts from HeLa, H-4-II-E, and COS-7 cells using #4827.*

# Sampler Kits




***Polycomb Group Antibody Sampler Kit #9788:*** *WB analysis of extracts from various cell lines using* ***(A) Ring1A Antibody #2820****,* ***(B) Ezh2 (D2C9) XP™ Rabbit mAb #5246****, and* ***(C) SUZ12 (D39F6) XP™ Rabbit mAb #3737****.*

| | | |
|---|---|---|
| | #9928 | Histone Deacetylase (HDAC) Antibody Sampler Kit |
| | #9933 | Acetyl-Histone Antibody Sampler Kit |
| | #9927 | Acetyl-Histone H3 Antibody Sampler Kit |
| | #9847 | Methyl-Histone H3 Antibody Sampler Kit |
| | #9849 | Phospho-Histone H3 (Mitotic Marker) Antibody Sampler Kit |
| NEW | #9788 | Polycomb Group Antibody Sampler Kit *featured* |

## What does Antibody Validation Mean at Cell Signaling Technology?

Scientists at Cell Signaling Technology (CST) follow a stringent validation protocol using a combination of several approaches and applications to provide you with the highest quality antibodies. This ensures credible and reproducible results with the least expenditure of your costly time, samples, and reagents.

### Antibody Validation at Cell Signaling Technology includes:

**Testing in a Number of Applications to help you choose the antibody that works best in your experiment.**

- Western blot, Immunoprecipitation, Immunohistochemistry, Immunofluorescence, Flow cytometry, ChIP, Sandwich ELISA

**Verifying Specificity and Reproducibility to ensure that the antibody performs consistently in all applications specified.**

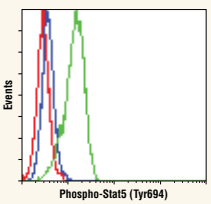
- Treatment of cells with appropriate kinase-specific inhibitors to verify specificity
- Analysis of a large panel of cell lines with known target expression levels to confirm target specificity
- Phosphatase treatment to verify phospho-specificity
- Comparison of antibody to isotype control antibody
- Verification of target-specific signal in transfected cells, knock-out cells, or siRNA-treated cells
- Blocking with antigen peptide
- Verification of correct subcellular localization or treatment-induced translocation
- Side-by-side comparison of a new lot with previous lots to ensure lot-to-lot consistency

**Identifying Optimal Conditions to save your precious time, samples, and reagents.**

- Optimal dilutions and buffers predetermined
- Positive and negative control cell extracts specified
- Detailed protocols already optimized

### Comparison of target-specific antibody to non-specific isotype control

**Phospho-Stat5 (Tyr694) (C71E5) Rabbit mAb #9314:** Flow cytometric analysis of K-562 cells, untreated (green) or gefitinib-treated (blue), using #9314 compared to concentration matched Rabbit (DA1E) mAb IgG XP™ Isotype Control #3900 (red).

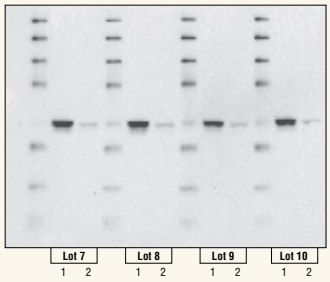



### Side by side comparison of new lot with previous lots

**Phospho-Akt (Ser473) Antibody #9271**

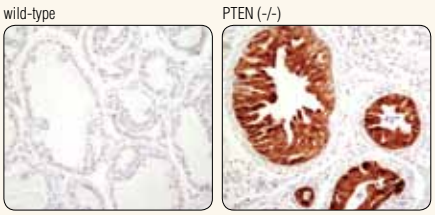
***Lot 7:*** *8/1/2002* ***Lot 9:*** *2/12/2004*
***Lot 8:*** *7/23/2003* ***Lot 10:*** *4/7/2006*

***1:*** *C2C12 cells +insulin(100 nM for 10 min.)*
***2:*** *C2C12 cells, untreated*

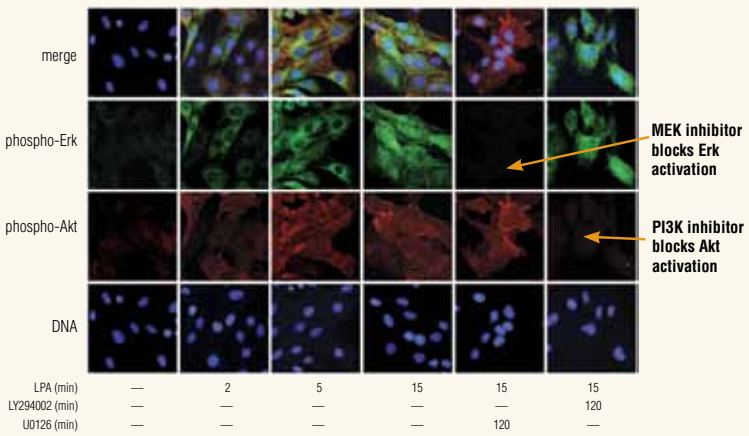



### Verification of target-specificity using mouse models

**Phospho-Akt (Ser473) (D9E) XP™ Rabbit mAb #4060:** IHC analysis of paraffin-embedded WT (left) and PTEN (-/-) (right) mouse prostate using #4060.
Tissue courtesy of Dr. David Guertin, The Whitehead Institute for Biomedical Research, Cambridge, MA.




### Verification of specificity using known target activators and inhibitors



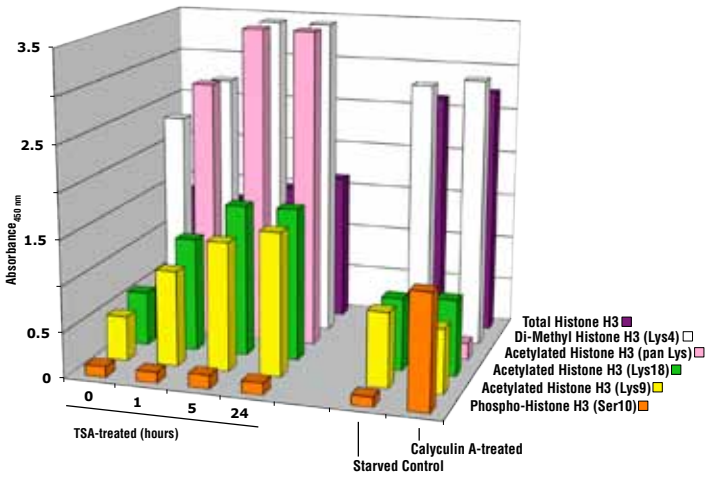



APPLICATIONS KEY:
**W** Western / **IP** Immunoprecipitation / **IHC** Immunohistochemistry / **IF** Immunofluorescence / **F** Flow Cytometry / **ChIP** Chromatin Immunoprecipitation / (**-IC** Immunocytochemistry, **-P** Paraffin, **-F** Frozen) / **E-P** Peptide ELISA

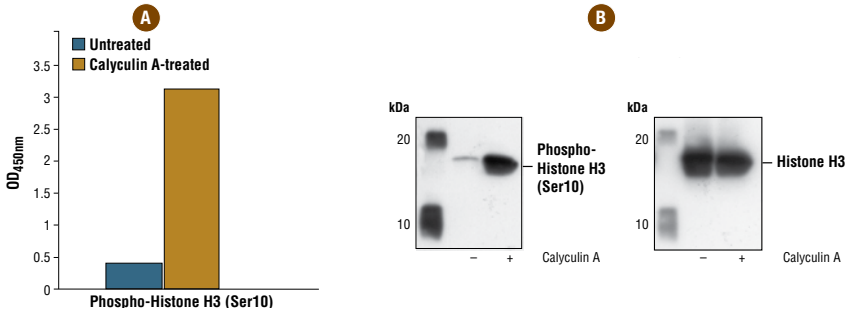
# PathScan® Sandwich ELISA

| | Reactivity |
|---|---|
| **#7233 PathScan® Acetyl-Histone H2A Sandwich ELISA Kit** | H, M, Mk |
| **#7218 PathScan® Acetyl-Histone H2B (Lys5) Sandwich ELISA Kit** | H, M, Mk |
| **#7222 PathScan® Acetyl-Histone H2B (Lys20) Sandwich ELISA Kit** | H, M, Mk |
| **#7178 PathScan® Acetyl-Histone H2B Sandwich ELISA Kit** | H, Mk |
| **#7155 PathScan® Phospho-Histone H3 (Ser10) Sandwich ELISA Kit** | H, M |
| **#7207 PathScan® Phospho-Histone H3 (Ser10) Sandwich ELISA Antibody Pair** | H, M |
| **#7123 PathScan® Mono-Methyl-Histone H3 (Lys4) Sandwich ELISA Kit** | H, M, Mk |
| **#7124 PathScan® Di-Methyl-Histone H3 (Lys4) Sandwich ELISA Kit** | H, M, Mk |
| **#7125 PathScan® Tri-Methyl-Histone H3 (Lys4) Sandwich ELISA Kit** | H, M, Mk |
| **#7862 PathScan® Di-Methyl-Histone H3 (Lys9) Sandwich ELISA Kit** | H, M, Mk |
| **#7864 PathScan® Pan-Methyl-Histone H3 (Lys9) Sandwich ELISA Kit** | H, M, Mk |
| **#7866 PathScan® Tri-Methyl-Histone H3 (Lys27) Sandwich ELISA Kit** | H, M, Mk |
| **#7868 PathScan® Di-Methyl-Histone H3 (Lys36) Sandwich ELISA Kit** | H, M, Mk |
| **#7121 PathScan® Acetyl-Histone H3 (Lys9) Sandwich ELISA Kit** | H, M, Mk |
| **#7122 PathScan® Acetyl-Histone H3 (Lys18) Sandwich ELISA Kit** | H, M, Mk |
| **#7232 PathScan® Acetylated Histone H3 Sandwich ELISA Kit** | H, M, Mk |
| **#7209 PathScan® Acetylated Histone H3 Sandwich ELISA Antibody Pair** | H, M, Mk |
| **#7253 PathScan® Total Histone H3 Sandwich ELISA Kit** | H, M, Mk |
| **#7224 PathScan® Acetyl-Histone H4 (Lys8) Sandwich ELISA Kit** | H, M, Mk |
| **#7228 PathScan® Acetyl-Histone H4 (Lys12) Sandwich ELISA Kit** | H, M, Mk |
| **#7238 PathScan® Acetyl-Histone H4 Sandwich ELISA Kit** | H, M, Mk |

Scientists at CST develop the highest quality research tools that are robust, sensitive, and specific. Our line of PathScan® Sandwich ELISA products provides researchers with a selection of assays for the study of critical regulatory proteins. In-house development, production, and validation of these kits ensure the highest possible product quality. Technical support is provided by the same scientists that produce the products, and know them best.




*COS cells were treated with either Trichostatin A #9950 or Calyculin A #9902 to inhibit HDACs or phosphatases, respectively. Histone acetylation or phosphorylation was analyzed using PathScan® Sandwich ELISA Kits: #7155, # 7121, #7122, #7232, #7124, #7253.*




◀ ***PathScan® Phospho-Histone H3 (Ser10) Sandwich ELISA Kit #7155:*** *Treatment of NIH/3T3 cells with Calyculin A #9902 causes accumulation of phospho-histone H3 (Ser10), detected by #7155, but does not affect the level of total histone H3 protein, detected by western analysis. $OD_{450nm}$ readings are shown in **(A)**, while the corresponding western blots using Phospho-Histone H3 (Ser10) Antibody #9701 (left panel) or Histone H3 Antibody #9715 (right panel), are shown in **(B)**.*

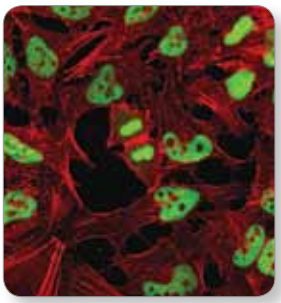
## Supporting Products

For your convenience, CST offers a wide selection of products to support your research needs. These products are the same used in-house for antibody validation in applications including western blotting, chromatin immunoprecipitation, immunohistochemistry, flow cytometry, and immunofluorescent analysis, and therefore work optimally with CST™ antibodies.

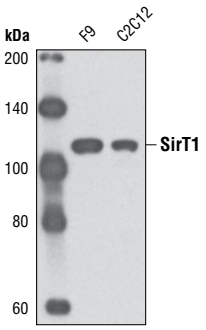
**Complementary Products include:**
- Growth Factors and Cytokines
- Inhibitors and Activators
- Secondary Antibodies
- Protein Markers
- Detection Systems
- Buffers
- Cellular Dyes

**Control Products include:**
- Cell Extracts
- Control Proteins
- Isotype Controls
- Blocking Peptides
- PCR Primers

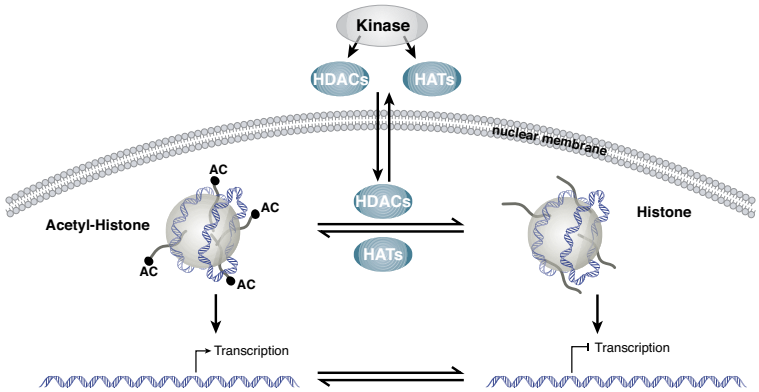
# Epigenetic Regulators

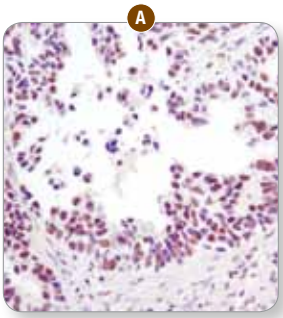


***HDAC1 (10E2) Mouse mAb #5356:*** *Confocal IF analysis of HeLa cells using #5356 (green). Actin filaments were labeled with DY-554 phalloidin (red).*

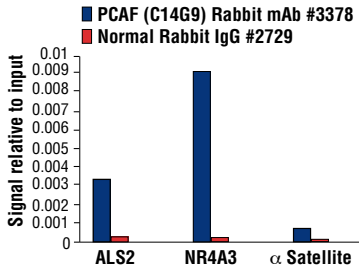



***SirT1 (D60E1) Rabbit mAb (Mouse Specific) #3931:*** *WB analysis of extracts from F9 and C2C12 cells using #3931.*




***Phospho-HDAC3 (S424) Antibody #3815: (A)*** *IHC analysis of paraffin-embedded human lung carcinoma using #3815.* ***(B)*** *WB analysis of extracts from HeLa, C6, and C2C12 cells, untreated or λ-phosphatase-treated, using #3815 (upper) or HDAC3 Antibody #2632 (lower).*




***PCAF (C14G9) Rabbit mAb #3378:*** *ChIP assays were performed with cross-linked chromatin from 4 x $10^6$ 293 cells treated with Forskolin #3828 (30 µM) and either 20 µl of #3378 or 2 µl of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using human ALS2 exon 1 primers, SimpleChIP® Human NR4A3 Promoter Primers #4829, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin (equivalent to one).*

**Selected Application References:**

**Histone Deacetylase 5 (HDAC5) Antibody #2082:**

Xia, S. et al. (2010) *Development* 137, 1075-1084. (W)

## Histone Acetylases and Deacetylases

| | | | Application | Reactivity |
|---|---|---|---|---|
| | #4771 | Acetyl-CBP (Lys1535)/p300 (Lys1499) Antibody | W, IP, ChIP | H, M, R, Mk |
| | #4772 | CBP Antibody | W | H, Mk |
| NEW | #5157 | CLOCK (D45B10) Rabbit mAb | W, IP | H, M, R, Mk |
| | #3305 | GCN5L2 (C26A10) Rabbit mAb | W, IP, IF-IC | H, M, R, Mk, (B) |
| | #2062 | Histone Deacetylase 1 (HDAC1) Antibody | W | H, M, R, Mk |
| NEW | #5356 | HDAC1 (10E2) Mouse mAb *featured* | W, IP, IF-IC | H, M, R, Mk |
| | #2540 | HDAC2 Antibody | W, IF-IC | H, M, R, Mk |
| | #2545 | HDAC2 Antibody (IP Preferred) | W, IP | H, M, Mk |
| NEW | #5113 | HDAC2 (3F3) Mouse mAb | W, IP, IF-IC | H, M, R, Mk |
| NEW | #3815 | Phospho-HDAC3 (Ser424) Antibody *featured* | W, IP, IHC-P, IF-IC | H, M, R, (Mk, C, X) |
| | #2632 | Histone Deacetylase 3 (HDAC3) Antibody | W | H, M, R, Mk |
| NEW | #3949 | HDAC3 (7G6C5) Mouse mAb | W, IP, IF-IC | H, M, R, Mk |
| | #3443 | Phospho-HDAC4 (Ser246)/HDAC5 (Ser259)/HDAC7 (Ser155) (D27B5) Rabbit mAb | W, IP | H, M |
| | #3424 | Phospho-HDAC4 (Ser632)/HDAC5 (Ser498)/HDAC7 (Ser486) Antibody | W, IP | H, M |
| | #2072 | Histone Deacetylase 4 (HDAC4) Antibody | W | H, M, R, Mk |
| NEW | #5392 | HDAC4 (4A3) Mouse mAb | W, IP | H, M, R, Mk |
| | #2082 | Histone Deacetylase 5 (HDAC5) Antibody | W, IP, IHC-P | H, M, R, Mk |
| | #2882 | Histone Deacetylase 7 (HDAC7) Antibody | W | H, M, R, Mk |
| | #3378 | PCAF (C14G9) Rabbit mAb *featured* | W, IP, ChIP | H, M, R, Mk, (B) |
| | #2327 | Phospho-SirT1 (Ser27) Antibody | W | H |
| | #2314 | Phospho-SirT1 (Ser47) Antibody | W, IP, IF-IC,F | H |
| NEW | #3931 | SirT1 (D60E1) Rabbit mAb (Mouse Specific) *featured* | W, IP | M |
| | #2496 | SirT1 (C14H4) Rabbit mAb | W, IP | H |
| | #2310 | SirT1 Antibody | W | H |
| | #2493 | SirT1 (D739) Antibody | W, IP, IF-IC | H, Mk |
| | #2028 | SirT1 Antibody (Mouse Specific) | W, IP, IF-IC | M |
| | #2313 | SirT2 Antibody | W | H, M, R, Mk |
| | #2627 | SirT3 (C73E3) Rabbit mAb | W, IP, IHC-P | H, R, Mk |
| | #2590 | SirT6 Antibody | W, IP, IF-IC | H |
| | #4562 | TFII-I Antibody | W, IP, IHC-P | H, M, Mk |
| NEW | #4169 | TFIIB (2F6A3H4) Mouse mAb | W, IP | H, M, R, Mk |
| | #3966 | TRRAP (D2966) Antibody | W, IP, IF-IC | H, M, R, Mk |
| | #3967 | TRRAP (P2032) Antibody | W, IP | H, M, Mk |
| | #2152 | WSTF Antibody | W, IP | H, M, R, Mk |
| | #9950 | Trichostatin A (TSA) | | |

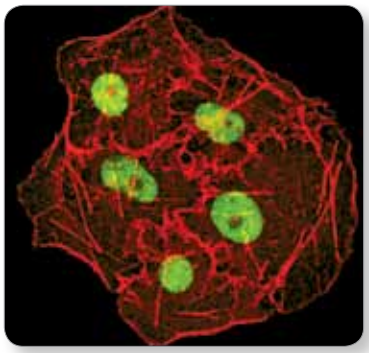


APPLICATIONS KEY:

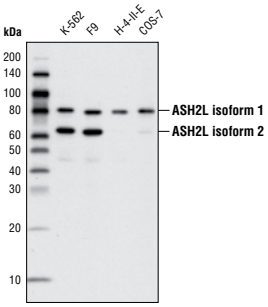
**W** Western / **IP** Immunoprecipitation / **IHC** Immunohistochemistry / **IF** Immunofluorescence / **F** Flow Cytometry / **ChIP** Chromatin Immunoprecipitation / (**-IC** Immunocytochemistry, **-P** Paraffin, **-F** Frozen) / **E-P** Peptide ELISA

## Methyltransferases and Demethylases

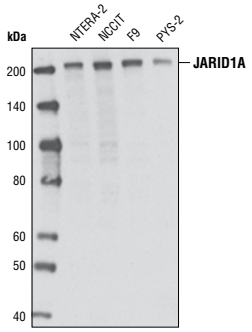
| | | | Application | Reactivity |
|---|---|---|---|---|
| NEW | #5019 | ASH2L (D93F6) XP™ Rabbit mAb *featured* | W, IP, IF-IC | H, M, R, Mk, (Dm) |
| NEW | #5032 | DNMT1 (D63A6) XP™ Rabbit mAb *featured* | W, IF-IC | H, M, R, Mk, (B) |
| NEW | #5119 | DNMT1 (D59A4) Rabbit mAb | W, IP | H, M, R, Mk, (B) |
| | #3598 | DNMT3A (D23G1) Rabbit mAb | W, IP | H, M, R, Mk, (B) |
| | #2160 | DNMT3A Antibody | W, IP | H, M, R, Mk, (B) |
| | #2161 | DNMT3B Antibody | W, IP | H, M, R, Mk, (Z) |
| | #2196 | ESET (C1C12) Rabbit mAb | W, IP, IF-IC | H, Mk |
| NEW | #5246 | Ezh2 (D2C9) XP™ Rabbit mAb *featured* | W, IP, IHC-P, IF-IC, ChIP | H, M, R, Mk |
| | #4905 | Ezh2 Antibody | W, IP, ChIP | H, M, R, Pg |
| | #3147 | Ezh2 (AC22) Mouse mAb | W, IF-IC | H, M, R, Mk |
| | #3306 | G9a/EHMT2 (C6H3) Rabbit mAb | W, IF-IC | H, M, R, Mk, (B, Pg) |
| NEW | #3876 | JARID1A (D28B10) XP™ Rabbit mAb *featured* | W, IP, IF-IC | H, M, (R, B) |
| | #3273 | JARID1B Antibody | W, IP | H, Mk |
| NEW | #5361 | JARID1C (D29B9) Rabbit mAb | W, IP | H, M, (Mk, Pg) |
| | #2621 | JMJD1B/JHDM2B Antibody | W, IP, IF-IC | H, M, R, Mk |
| | #3314 | JMJD1B (C69G2) Rabbit mAb | W, IP, IF-IC | H, Mk |
| | #3100 | JMJD1B (C6D12) Rabbit mAb | W, IP, IHC-P | H, Mk |
| NEW | #5377 | JMJD1B (6A1-1F5) Mouse mAb | W, IP, IF-IC | H, M, R, Mk |
| | #3393 | JMJD2A (C70G6) Rabbit mAb | W, IP, IF-IC | H, M, R, (Mk) |
| | #2898 | JMJD2B Antibody | W, IP | H, (Mk) |
| | #3457 | JMJD3 Antibody | W | H, M, (Mk) |
| | #2184 | LSD1 (C69G12) Rabbit mAb | W, IP, IHC-P, IHC-F, IF-IC | H, M, R, Mk |
| | #2139 | LSD1 Antibody | W, IP, IHC-P, IF-IC, F | H, M, R, Mk |
| | #4064 | LSD1 (1B2E5) Mouse mAb | W | H, M, R, Mk |
| NEW | #4218 | LSD1 (1E5-H2) Mouse mAb | W, IP | H, Mk |
| | #2018 | MEP50 (D56B8) Rabbit mAb | W, IP | H, M, R, Mk |
| | #2828 | MEP50 (P328) Antibody | W, IP | H |
| | #2823 | MEP50 Antibody | W, IP, IF-IC | H |
| NEW | #4789 | Nucleomethylin Antibody | W | H |
| | #2449 | PRMT1 (A33) Antibody | W, IP, IF-IC | H, M, R, Mk, (B) |
| | #2453 | PRMT1 (F339) Antibody | W | H, M, R, Mk, (B) |
| | #3379 | PRMT4/CARM1 (C31G9) Rabbit mAb | W, IP, IF-IC | H, M, R, Mk |
| | #4438 | PRMT4/CARM1 Antibody | W, IP | H, M, R, Mk |
| | #2252 | PRMT5/Skb1Hs Methyltransferase Antibody | W, IP | H, M, R, Mk |
| | #2825 | SET7/SET9 (C24B1) Rabbit mAb | W | H, M, R, Mk |
| | #2813 | SET7/SET9 Antibody | W, IF-IC | H, M, R, Mk |
| | #2996 | SET8 (C18B7) Rabbit mAb | W, IF-IC | H, M, R, Mk, (B, Pg) |
| NEW | #4251 | SMYD2 Antibody | W | H, M, R, Mk, (B, Pg) |
| | #2991 | SUV39H1 Histone Methyltransferase Antibody | W | H, M |
| | #3737 | SUZ12 (D39F6) XP™ Rabbit mAb | W,IP, IF-IC, ChIP | H, M, R, Mk |



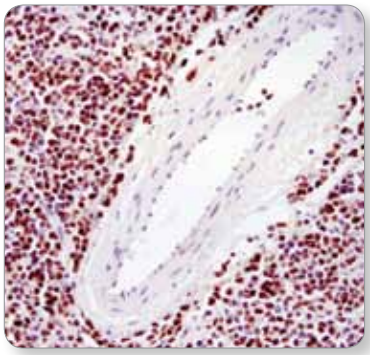
***DNMT1 (D63A6) XP™ Rabbit mAb #5032:*** *Confocal IF analysis of COS-7 cells using #5032 (green). Actin filaments were labeled using DY-554 phalloidin (red).*




***ASH2L (D93F6) XP™ Rabbit mAb #5019:*** *WB analysis of extracts from various cell lines using #5019.*




***JARID1A (D28B10) XP™ Rabbit mAb #3876:*** *WB analysis of extracts from various cell lines using #3876.*



***Ezh2 (D2C9) XP™ Rabbit mAb #5246:*** *IHC analysis of paraffin-embedded human lymphoma using #5246.*

**Selected Application References:**

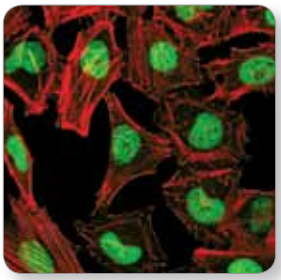
**Ezh2 (AC22) Mouse mAb #3147:**

Kalushkova, A. et al. (2010) *PLoS One* 5, e11483. (W)

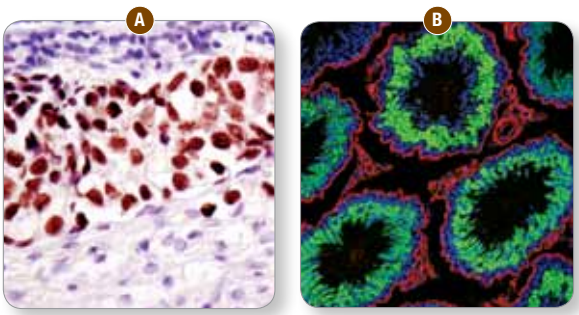
Van Dessel, N. et al. (2010) *Nucleic Acids Res.*, in press. (W)

**UNPARALLELED PRODUCT QUALITY, VALIDATION, AND TECHNICAL SUPPORT**

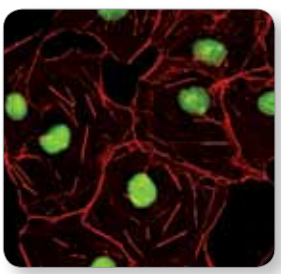
# HP1 and Other Chromatin Proteins



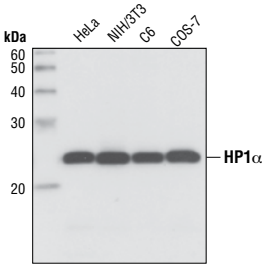
◀ ***CHAF1A (D77D5) XP™ Rabbit mAb #5480:*** *Confocal IF analysis of HeLa cells using #5480 (green). Actin filaments were labeled with DY-554 phalloidin (red).*



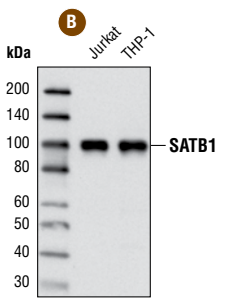

***NUT (C52B1) Rabbit mAb #3625: (A)*** *IHC analysis of paraffin-embedded human midline carcinoma using #3625.* ***(B)*** *Confocal IF analysis of rat testes using #3625 (green) and Pan-Keratin (C11) Mouse mAb #4545 (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).*



***HMGB1 Antibody #3935:*** *Confocal IF analysis of COS-7 cells using #3935 (green). Actin filaments were labeled using DY-554 phalloidin (red).*




***HP1α (C7F11) Rabbit mAb #2623:*** *WB analysis of extracts from HeLa, NIH/3T3, C6, and COS-7 cells using #2623.*


***SATB1 (L745) Antibody #3650: (A)*** *Confocal IF analysis of 293 cells using #3650 (green). Actin filaments were labeled with DY-554 phalloidin (red).* ***(B)*** *WB analysis of extracts from Jurkat and THP-1 cell lines using #3650.*

## HP1

| | | Application | Reactivity |
|---|---|---|---|
| | **#2623 HP1α (C7F11) Rabbit mAb** *featured* | W, IP, IHC-P, IF-IC | H, M, R, Mk |
| | **#2616 HP1α Antibody** | W, IP, IHC-P, IF-IC, F | H, M, R, Mk, (B) |
| | **#2613 HP1β Antibody** | W | H, M, R, Mk, (B) |
| | **#2600 Phospho-HP1γ (Ser83) Antibody** | W, IP, IF-IC | H, M, R, Mk, (Dm, B) |
| | **#2619 HP1γ Antibody** | W, IP, IF-IC, F | H, M, R, Mk |

## Other Chromatin Proteins

| | | | |
|---|---|---|---|
| | **#2990 ASF1A (C6E10) Rabbit mAb** | W, IP, IHC-P, IF-IC | H, M, Mk, (C, B) |
| | **#2902 ASF1B (C70E2) Rabbit mAb** | W, IP, IF-IC | H, Mk |
| | **#2769 ASF1B Antibody** | W, IP | H, Mk, (M) |
| | **#2830 Bmi1 Antibody** | W, IF-IC | H, R, Mk, (B) |
| | **#3508 Brg1 (A52) Antibody** | W, IF-IC | H, M, Mk, (R) |
| | **#3514 Brg1 (P680) Antibody** | W | H, M, R, Mk |
| NEW | **#5480 CHAF1A (D77D5) XP™ Rabbit mAb** *featured* | W, IP, IF-IC | H, Mk |
| NEW | **#4170 CHD2 Antibody** | W | H, M, R, Mk |
| NEW | **#4241 CHD3 Antibody** | W, IP | H, M, Mk |
| NEW | **#4245 CHD4 Antibody** | W | H, M, R, (Mk, B) |
| | **#3417 CTCF (D1A7) XP™ Rabbit mAb** | W, IP, IF-IC, ChIP | H, R, Mk, (B) |
| | **#3418 CTCF (D31H2) XP™ Rabbit mAb** | W, IP, IHC-P, IF-IC, ChIP | H, M, R, Mk, (B) |
| | **#2899 CTCF Antibody** | W, IP, IF-IC, ChIP | H, M, R, Mk |
| NEW | **#4880 Phospho-DBC1 (Thr454) Antibody** | W, IP, IF-IC | H |
| NEW | **#5693 DBC1 Antibody** | W, IP | H, M, R, Mk |
| NEW | **#5269 HMGA2 Antibody** | W, IP | H, M, R |
| NEW | **#3935 HMGB1 Antibody** *featured* | W, IF-IC | H, M, R, Mk, (Hm, B, Pg) |
| | **#2088 LEDGF (C57G11) Rabbit mAb** | W, IHC-P, IF-IC, F | H, M, R, (Mk) |
| | **#3896 MBD3 Antibody** | W | H, M, R, Mk |
| | **#3456 MeCP2 (D4F3) XP™ Rabbit mAb** | W, IP, IHC-P, IF-IC | H, M, R, Mk |
| NEW | **#3625 NUT (C52B1) Rabbit mAb** *featured* | W, IP, IHC-P, IF-F | H, R, (Mk) |
| NEW | **#3497 PHF2 (D45A2) Rabbit mAb** | W, IP | H, M, R, Mk |
| NEW | **#3934 PHF20 (D96F6) XP™ Rabbit mAb** | W, IP, IF-IC | H, M, R, Mk, (B) |
| NEW | **#4522 RBAP46 Antibody** | W | H, M, R, Mk, (B) |
| NEW | **#4633 RBAP46/RBAP48 Antibody** | W | H, M, R, Mk, (C) |
| | **#2820 Ring1A Antibody** | W | H, M, R, Mk |
| NEW | **#5694 RING1B (D22F2) XP™ Rabbit mAb** | W, IP, IF-IC, ChIP | H, M, R, Mk |
| NEW | **#4028 Phospho-SATB1 (Ser47) Antibody** | W | H, M, R, (Mk, B) |
| NEW | **#3650 SATB1 (L745) Antibody** *featured* | W, IP, IF-IC | H, (Mk) |
| NEW | **#3643 SATB1 (P472) Antibody** | W, IP | H, M, R, (Mk, B, Pg) |
| NEW | **#5329 SMC2 (D23C5) Rabbit mAb** | W, IP | H, M, R, Mk |
| NEW | **#5394 SMC2 (D91E3) Rabbit mAb** | W, IP | H, R, Mk |
| NEW | **#5696 SMC3 (D47B5) Rabbit mAb** | W, IP, IF-IC | H, M, R, Mk |
| NEW | **#5547 SMC4 (D14E2) Rabbit mAb** | W, IP | H, M, R, Mk, (X, B) |
| NEW | **#4239 STAG2 Antibody** | W, IP | H, M, R, Mk |
| NEW | **#5433 TERF2IP (D9H4) Rabbit mAb** | W, IP | H, M, R, Mk |
| | **#4733 Topoisomerase IIα Antibody** | W, IP, F | H, M, R, Mk |

**Selected Application References:**

**ASF1B (C70E2) Rabbit mAb #2902:**
Jasencakova, Z. et al. (2010) *Mol. Cell* 37, 736-743. (IF-IC)

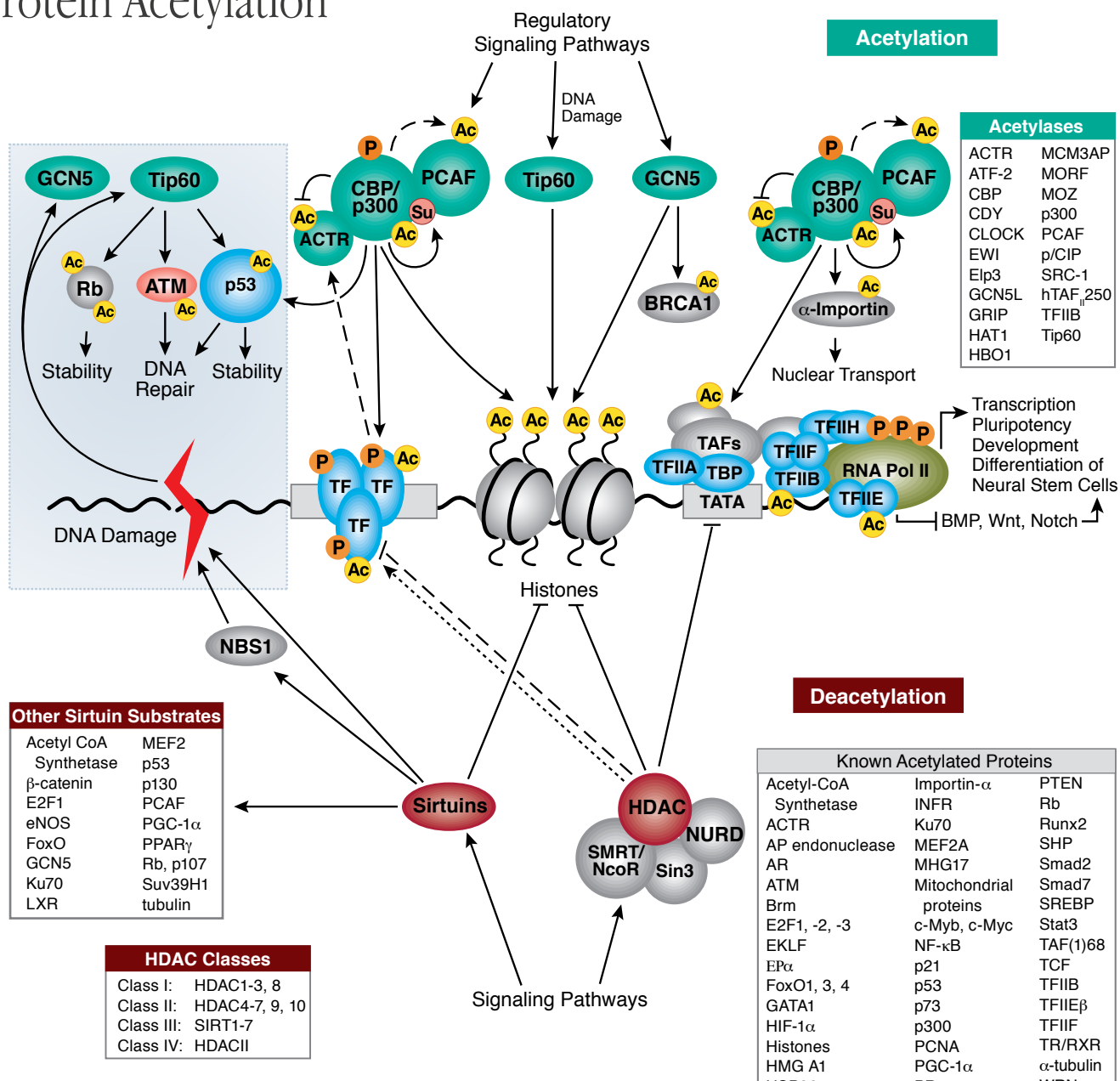
**NUT (C52B1) Rabbit mAb #3625:**
Haack, H. et al. (2009) *Am. J. Surg. Pathol.* 33, 984-991. (IHC-P)



APPLICATIONS KEY: **W** Western / **IP** Immunoprecipitation / **IHC** Immunohistochemistry / **IF** Immunofluorescence / **F** Flow Cytometry / **ChIP** Chromatin Immunoprecipitation / (**-IC** Immunocytochemistry, **-P** Paraffin, **-F** Frozen) / **E-P** Peptide ELISA
REACTIVITY KEY: **H** human / **M** mouse / **R** rat / **Hm** hamster / **Mk** monkey / **C** chicken / **Mi** mink / **Dm** D. melanogaster / **X** Xenopus / **Z** zebra fish / **B** bovine / **Dg** dog / **Pg** pig / **Sc** S. cerevisiae / **All** all species expected / **( )** 100% sequence homology

# Signaling Pathways

## Protein Acetylation






**Pathway Description:** Protein acetylation plays a crucial role in regulating chromatin structure and transcriptional activity. Many transcriptional coactivators possess intrinsic acetylase activity, while transcriptional corepressors are associated with deacetylase activity. Acetylation complexes (such as CBP/p300 and PCAF) or deacetylation complexes (such as Sin3, NuRD, NcoR and SMRT) are recruited to DNA-bound transcription factors (TFs) in response to signaling pathways. Histone hyperacetylation by histone acetyltransferases (HATs) is associated with transcriptional activation, whereas histone deacetylation by histone deacetylases (HDACs) is associated with transcriptional repression. Histone acetylation stimulates transcription by remodeling higher order chromatin structure, weakening histone-DNA interactions, and providing binding sites for transcriptional activation complexes containing proteins that possess bromodomains, which bind acetylated lysine. Histone deacetylation represses transcription through an inverse mechanism involving the assembly of compact higher order chromatin and the exclusion of bromodomain-containing transcription activation complexes. Histone hypoacetylation is a hallmark of silent heterochromatin. Site-specific acetylation of a growing number of non-histone proteins, including p53 and E2F, has been shown to regulate their activity, localization, specific interactions, and stability/degradation, therefore controlling a variety of cellular processes, such as transcription, proliferation, apoptosis, and differentiation. At an organismal level, acetylation plays an important role in immunity, circadian rhythmicity, and memory formation. Protein acetylation is becoming a favorable target in drug design for numerous disease conditions.

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Direct Inhibitory Modification
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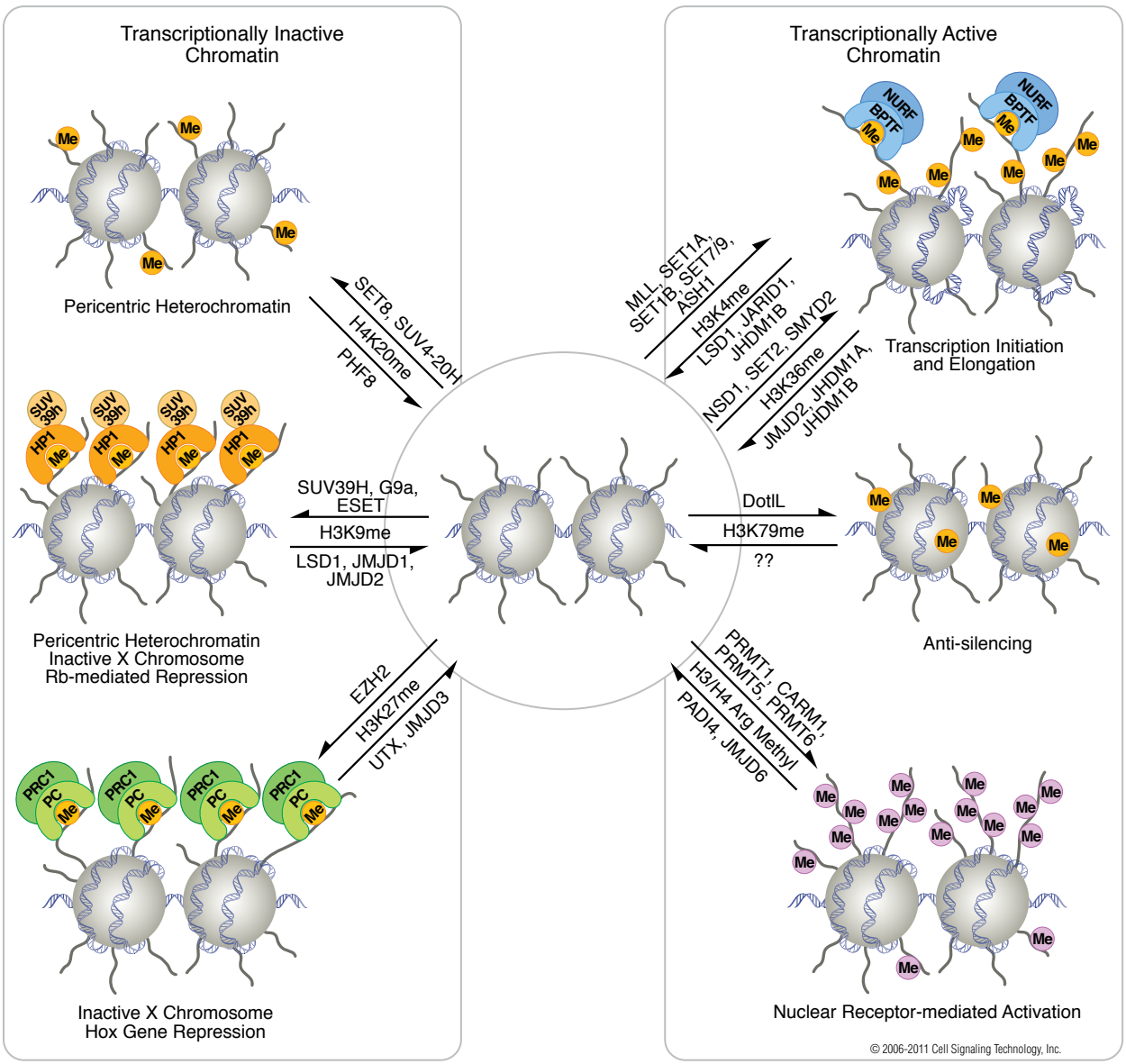
Phosphatase
Transcription Factor
Caspase
Receptor
Enzyme
Cyclin, pro-apoptotic
pro-survival
GAP
GEF
GTPase
G-protein, ribosomal subunit

# Histone Methylation




**Pathway Description:** The nucleosome, made up of four histone proteins (H2A, H2B, H3, and H4), is the primary building block of chromatin. Originally thought to function as a static scaffold for DNA packaging, histones have more recently been shown to be dynamic proteins, undergoing multiple types of post-translational modifications. Two such modifications, methylation of arginine and lysine residues are major determinants for formation of active and inactive regions of the genome. Arginine methylation of histones H3 (Arg2, 17, 26) and H4 (Arg3) promotes transcriptional activation and is mediated by a family of protein arginine methyltransferases (PRMTs), including the co-activators PRMT1 and CARM1 (PRMT4). In contrast, a more diverse set of histone lysine methyltransferases has been identified, all but one of which contain a conserved catalytic SET domain originally identified in the Drosophila Su[var]3-9, Enhancer of zeste, and Trithorax proteins. Lysine methylation has been implicated in both transcriptional activation (H3 Lys4, 36, 79) and silencing (H3 Lys9, 27, H4 Lys20).

Unlike acetylation, methylation does not alter the charge of arginine and lysine residues and is unlikely to directly modulate nucleosomal interactions required for chromatin folding. While the mechanisms by which arginine methylation regulates transcription are unknown, lysine methylation coordinates the recruitment of chromatin modifying enzymes. Chromodomains (HP1, PRC1), PHD fingers (BPTF, ING2), Tudor domains (53BP1), and WD-40 domains (WDR5) are among a growing list of methyl-lysine binding modules found in histone acetyltransferases, deacetylases, methylases and ATP-dependent chromatin remodeling enzymes. Lysine methylation provides a binding surface for these enzymes, which then regulate chromatin condensation and nucleosome mobility in order to maintain local regions of active or inactive chromatin. In addition, lysine methylation can block binding of proteins that interact with unmethylated histones or directly inhibit catalysis of other regulatory modifications on neighboring residues. The presence of methyl-lysine binding modules in the DNA repair protein 53BP1 suggests roles for lysine methylation in other cellular processes.

Histone methylation is crucial for proper programming of the genome during development and misregulation of the methylation machinery can lead to diseased states such as cancer. Until recently, methylation was believed to be an irreversible, stable epigenetic mark that is propagated through multiple cell divisions, maintaining a gene in an active or inactive state. While there is no argument that methylation is a stable mark, recent identification of histone demethylases such as LSD1/AOF2, JMJD1, JMJD2 and JHDM1 has shown that methylation is reversible and provides a rational for how genomes might be reprogrammed during differentiation of individual cell lineages.

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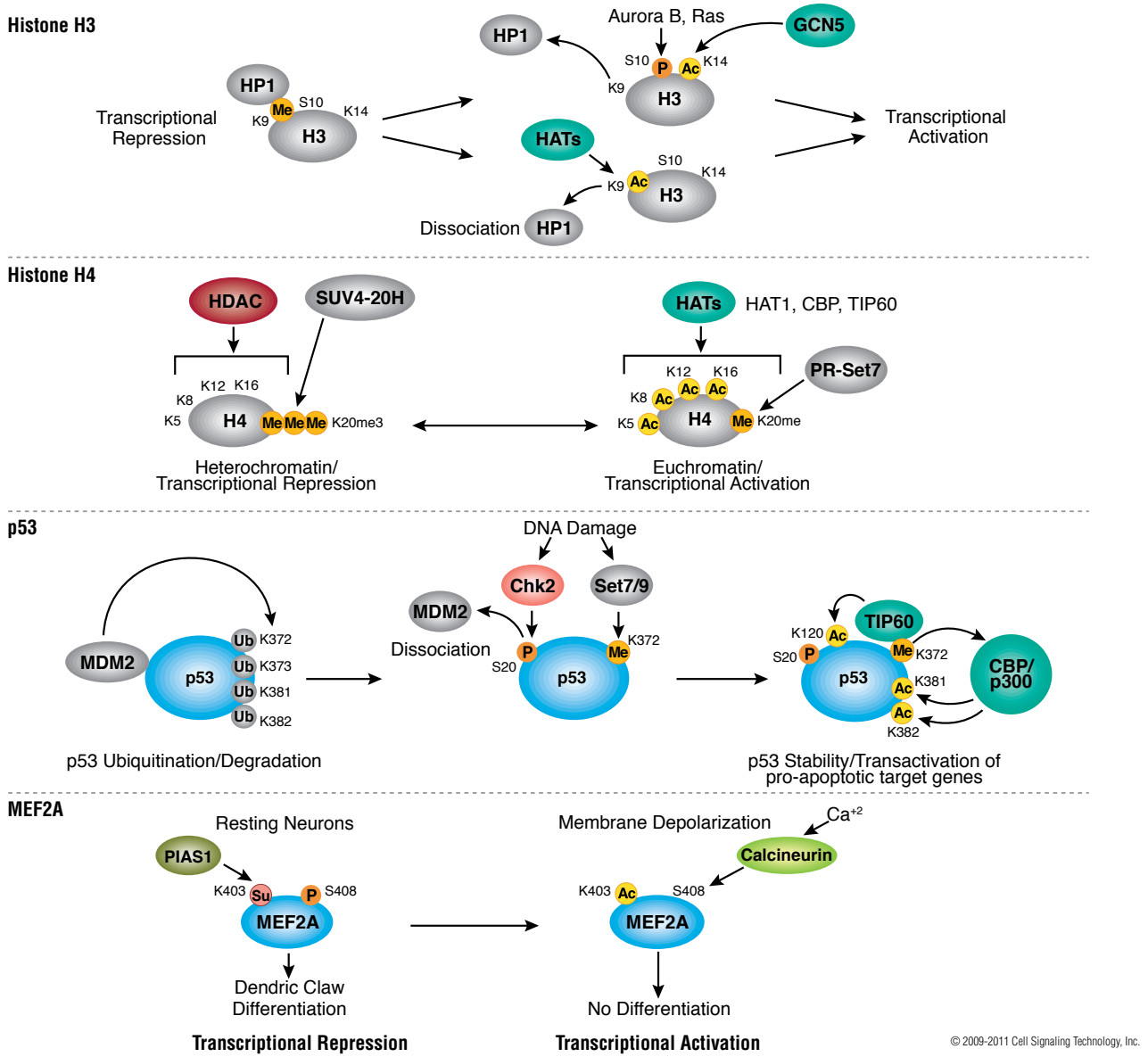
# Examples of Crosstalk Between Post-translational Modifications






**Pathway Description:** Post-translational modifications (PTMs) have recently emerged as major regulators of protein function. Originally described in histones, these various chemical modifications (methylation, acetylation, phosphorylation, sumoylation, and more) have now been identified in non-histone proteins as well. Early work defined a putative role for each of these modifications, for instance, acetylation correlates with activation and methylation with repression. However, more recent studies indicate that some of these modifications could trigger either activation or silencing in a context dependent manner. For instance, methylation of histone H3 Lys9 correlates with repression, while methylation of H3 Lys4 correlates with activation. Furthermore, each of these moieties can be either mono-, di- or tri-methylated, and depending on the degree of methylation, the biological output will be completely different. Until recently, PTMs were considered independently, under the assumption that their functions would not be related to one another. It is now clear that PTMs work in concert, and the crosstalk between different modifications determines the final biological read-out. In this context, some modifications can influence others, and it appears that specific combinations of these modifications can form a dynamic "code". We provide a few examples of this type of crosstalk above. Although each of the modifications shown here are occurring in cis, there are now clear examples, at least for histones, where modifications in one histone molecule can regulate modifications in other histones in trans. Although there are now many examples of these "functional networks", it is likely that we have just begun to scratch the surface. Better antibodies and novel technologies will help to complete this crosstalk puzzle, for which the specific fine-tuning appears critical to determine life as we know it.

**Selected Reviews:**

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Berger, S.L. (2007) The complex language of chromatin regulation during transcription. *Nature* 447, 407–412.

# Histone Modification Table

## Acetylation

| Histone | Site | Histone-modifying Enzymes | Proposed Function | Ref. # |
|---|---|---|---|---|
| H2A | Lys4 (S. cerevisiae) | Esa1 | transcriptional activation | (1) |
| | Lys5 (mammals) | Tip60, p300/CBP | transcriptional activation | (2,3) |
| | Lys7 (S. cerevisiae) | Hat1 | unknown | (4) |
| | | Esa1 | transcriptional activation | (1) |
| H2B | Lys5 | p300, ATF2 | transcriptional activation | (3,5) |
| | Lys11 (S. cerevisiae) | Gcn5 | transcriptional activation | (6) |
| | Lys12 (mammals) | p300/CBP, ATF2 | transcriptional activation | (3,5) |
| | Lys15 (mammals) | p300/CBP, ATF2 | transcriptional activation | (3,5) |
| | Lys16 (S. cerevisiae) | Gcn5, Esa1 | transcriptional activation | (6) |
| | Lys20 | p300 | transcriptional activation | (3) |
| H3 | Lys4 (S. cerevisiae) | Esa1 | transcriptional activation | (1) |
| | | Hpa2 | unknown | (7) |
| | Lys9 | unknown | histone deposition | (8) |
| | | Gcn5, SRC-1 | transcriptional activation | (9,10) |
| | Lys14 | unknown | histone deposition | (8) |
| | | Gcn5, PCAF | transcriptional activation | (3,11) |
| | | Esal, Tip60 | transcriptional activation | (1,2) |
| | | | DNA repair | (11,12) |
| | | SRC-1 | transcriptional activation | (10) |
| | | Elp3 | transcriptional activation (elongation) | (13) |
| | | Hpa2 | unknown | (7) |
| | | hTFIIIC90 | RNA polymerase III transcription | (14) |
| | | TAF1 | RNA polymerase II transcription | (15) |
| | | Sas2 | euchromatin | (16) |
| | | Sas3 | transcriptional activation (elongation) | (17) |
| | | p300 | transcriptional activation | (3) |
| | Lys18 | Gcn5 | transcriptional activation, DNA repair | (9) |
| | | p300/CBP | DNA replication, transcriptional activation | (3,18) |
| | Lys23 | unknown | histone deposition | (8) |
| | | Gcn5 | transcriptional activation, DNA repair | (9) |
| | | Sas3 | transcriptional activation (elongation) | (17) |
| | | p300/CBP | transcriptional activation | (3,18) |
| | Lys27 | Gcn5 | transcriptional activation | (6) |
| | Lys56 (S. cerevisiae) | Spt10 | transcriptional activation | (19) |
| | | | DNA repair | (20) |
| H4 | Lys5 | Hat1 | histone deposition | (21) |
| | | Esal, Tip60 | transcriptional activation | (1,2) |
| | | | DNA repair | (11,12) |
| | | ATF2 | transcriptional activation | (5) |
| | | Hpa2 | unknown | (7) |
| | | p300 | transcriptional activation | (3) |
| | Lys8 | Gcn5, PCAF | transcriptional activation | (3,22) |
| | | Esal, Tip60 | transcriptional activation | (1,2) |
| | | | DNA repair | (11,12) |
| | | ATF2 | transcriptional activation | (5) |
| | | Elp3 | transcriptional activation (elongation) | (13) |
| | | p300 | transcriptional activation | (3) |
| | Lys12 | Hat1 | histone deposition | (21) |
| | | | telomeric silencing | (23) |
| | | Esal, Tip60 | transcriptional activation | (1,2) |
| | | | DNA repair | (11,12) |
| | | Hpa2 | unknown | (7) |
| | | p300 | transcriptional activation | (3) |
| | Lys16 | Gcn5 | transcriptional activation | (22) |
| | | MOF (D. melanogaster) | transcriptional activation | (24) |
| | | Esal, Tip60 | transcriptional activation | (1,2) |
| | | | DNA repair | (11,12) |
| | | ATF2 | transcriptional activation | (5) |
| | | Sas2 | euchromatin | (2,6) |
| | Lys91 (S. cerevisiae) | Hat1/Hat2 | chromatin assembly | (25) |

## Methylation

| Histone | Site | Histone-modifying Enzymes | Proposed Function | Ref. # |
|---|---|---|---|---|
| H1 | Lys26 | Ezh2 | transcriptional silencing | (48,49) |
| H3 | Lys4 | Set1 (S. cerevisiae) | permissive euchromatin (di-Me) | (26) |
| | | Set7/9 (vertebrates) | transcriptional activation (tri-Me) | (27) |
| | | MLL, ALL-1 | transcriptional activation | (28,29) |
| | | Ash1 (D. melanogaster) | transcriptional activation | (30) |
| | Arg8 | PRMT5 | transcriptional repression | (31) |
| | Lys9 | Suv39h,Clr4 | transcriptional silencing (tri-Me) | (32,33) |
| | | G9a | transcriptional repression genomic imprinting | (34) |
| | | SETDB1 | transcriptional repression (tri-Me) | (35) |
| | | Dim-5 (N.crassa), Kryptonite (A. thaliana) | DNA methylation (tri-Me) | (36,37) |
| | | Ash1 (D. melanogaster) | transcriptional activation | (30) |
| | Arg17 | CARM1 | transcriptional activation | (18) |
| | Lys27 | Ezh2 | transcriptional silencing | (38) |
| | | | X inactivation (tri-Me) | |
| | | G9a | transcriptional silencing | (34) |
| | Lys36 | Set2 | transcriptional activation (elongation) | (39) |
| | Lys79 | Dot1 | euchromatin | (40) |
| | | | transcriptional activation (elongation) | (41) |
| | | | checkpoint response | (42) |
| H4 | Arg3 | PRMT1 | transcriptional activation | (43) |
| | | PRMT5 | transcriptional repression | (31) |
| | Lys20 | PR-Set7 | transcriptional silencing (mono-Me) | (44) |
| | | Suv4-20h | heterochromatin (tri-Me) | (45) |
| | | Ash1 (D. melanogaster) | transcriptional activation | (30) |
| | | Set9 (S. pombe) | checkpoint response | (46) |
| | Lys59 | unknown | transcriptional silencing | (47) |

## Phosphorylation

| Histone | Site | Histone-modifying Enzymes | Proposed Function | Ref. # |
|---|---|---|---|---|
| H1 | Ser27 | unknown | transcriptional activation, chromatin decondensation | (48,49) |
| H2A | Ser1 | unknown | mitosis, chromatin assembly | (50) |
| | | MSK1 | transcriptional repression | (51) |
| | Thr119 (D. melanogaster) | NHK1 | mitosis | (52) |
| | Ser122 (S. cerevisiae) | unknown | DNA repair | (53) |
| | Ser129 (S. cerevisiae) | Mec1, Tel1 | DNA repair | (54,55) |
| | Ser139 (mammalian H2A.X) | ATR, ATM, DNA-PK | DNA repair | (56-58) |
| H2B | Ser10 (S. cerevisiae) | Ste20 | apoptosis | (59) |
| | Ser14 (vertebrates) | Mst1 | apoptosis | (60) |
| | | unknown | DNA repair | (61) |
| | Ser33 (D. melanogaster) | TAF1 | transcriptional activation | (62) |
| H3 | Thr3 | Haspin/Gsg2 | mitosis | (63) |
| | Ser10 | Aurora-B kinase | mitosis, meiosis | (64,65) |
| | | MSK1, MSK2 | immediate-early gene activation | (66) |
| | | IKK-α | transcriptional activation | (67) |
| | | Snf1 | transcriptional activation | (68) |
| | Thr11 (mammals) | Dlk/Zip | mitosis | (69) |
| | Ser28 (mammals) | Aurora-B kinase | mitosis | (70) |
| | | MSK1, MSK2 | immediate-early activation | (66,71) |
| H4 | Ser1 | unknown | mitosis, chromatin assembly | (50) |
| | | CK2 | DNA repair | (72) |

## Ubiquitylation

| Histone | Site | Histone-modifying Enzymes | Proposed Function | Ref. # |
|---|---|---|---|---|
| H2A | Lys119 (mammals) | Ring2 | spermatogenesis | (73) |
| H2B | Lys120 (mammals) | UbcH6 | meiosis | (74) |
| | Lys123 (S. cerevisiae) | Rad6 | transcriptional activation euchromatin | (75) |

## Sumoylation

| Histone | Site | Histone-modifying Enzymes | Proposed Function | Ref. # |
|---|---|---|---|---|
| H2A | Lys126 (S. cerevisiae) | Ubc9 | transcriptional repression | (76) |
| H2B | Lys6 or Lys7 (S. cerevisiae) | Ubc9 | transcriptional repression | (76) |
| H4 | N-terminal tail (S. cerevisiae) | Ubc9 | transcriptional repression | (77) |

## Biotinylation

| Histone | Site | Histone-modifying Enzymes | Proposed Function | Ref. # |
|---|---|---|---|---|
| H2A | Lys9 | biotinidase | unknown | (78) |
| | Lys13 | biotinidase | unknown | (78) |
| H3 | Lys4 | biotinidase | gene expression | (79) |
| | Lys9 | biotinidase | gene expression | (79) |
| | Lys18 | biotinidase | gene expression | (79) |
| H4 | Lys12 | biotinidase | DNA damage response | (80,81) |

**Histone Modification Table Description:** The nucleosome, made up of four core histone proteins (H2A, H2B, H3, and H4), and linker histone H1 are the primary building blocks of chromatin. Originally thought to function as a static scaffold for DNA packaging, histones have more recently been shown to be dynamic proteins, undergoing multiple types of post-translational modifications that regulate chromatin condensation and DNA accessibility. For example, acetylation of lysine residues has long been associated with histone deposition and transcriptional activation, and more recently found to be associated with DNA repair. Phosphorylation of serine and threonine residues facilitates chromatin condensation during mitosis and transcriptional activation of immediate-early genes. Methylation of lysine and arginine residues function as a major determinant for formation of transcriptionally active and inactive regions of chromatin and is crucial for proper programming of the genome during development. This table provides a referenced list of many known histone modifications, the associated modifying enzymes, and proposed functions.

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Printed in the USA on recycled paper (25% post-consumer waste fiber) using vegetable inks and processed chlorine free.