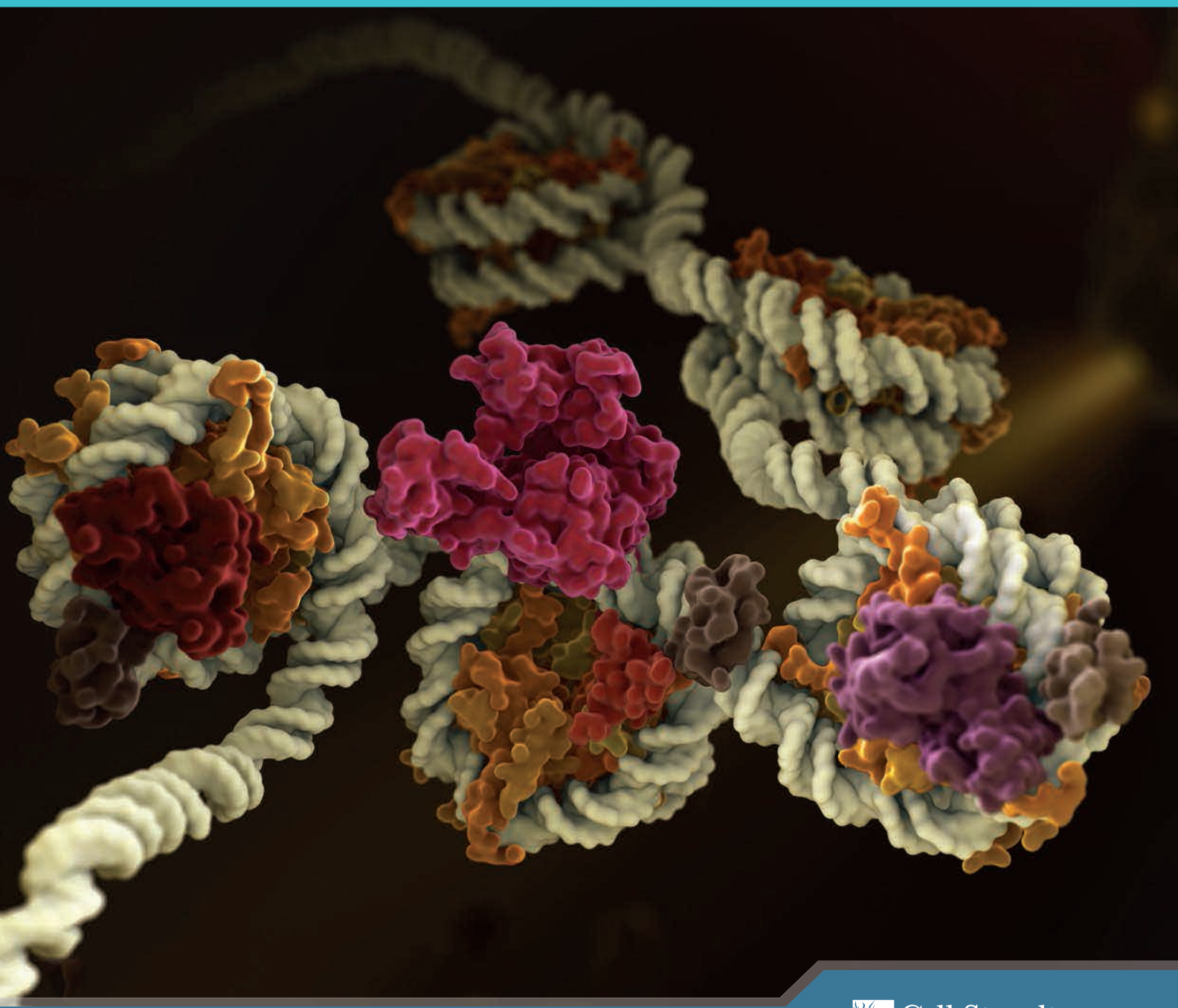
CELL SIGNALING TECHNOLOGY

Antibodies, Kits, and Reagents for

Epigenetics and Chromatin Regulation



Cell Signaling TECHNOLOGY®

Chris, Sr. Group Leader, Development
has been with CST since 2005.

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Epigenetics and Chromatin Regulation

Epigenetics describes changes in gene expression passed from one cellular generation to the next that occur without a corresponding change to the DNA sequence. In such cases, gene expression is generally determined by modifications at the chromatin level.

The basic unit of chromatin is the nucleosome, which comprises 146 base pairs of DNA wound around a core of histone proteins (a histone octamer) and epigenetic modifications may be made to either the DNA or the protein core component. The DNA component is commonly modified via methylation at CpG dinucleotides. This modification generally occurs in a bimodal pattern, such that CpG dinucleotides are largely methylated across the genome except for when they are clustered together into sparsely methylated CpG islands. Modifications to the protein core component include acetylation, methylation, ubiquitylation, and phosphorylation. These post-translational modifications may occur in the N-terminal and C-terminal tails and/or the core of each histone protein.

Epigenetic modifications facilitate remodeling of the chromatin to make the DNA more or less accessible to the transcriptional machinery. For example, methylation of the DNA facilitates heterochromatin formation and gene silencing, whereas histone acetylation is generally thought to relax chromatin structure and facilitate gene transcription. Accordingly, mutations in genes associated with epigenetic maintenance have been linked to a diverse set of pathologies from neurological, metabolic, and cardiac diseases to cancer. As a result, the study of epigenetics and chromatin regulation has become an important focus for basic and clinical researchers alike.

Selected Reviews: Binda, O. (2013) *Epigenetics* 8, 457–463. | Lahue, R.S. and Frizzell, A. (2012) *Epigenetics* 7, 806–810. | Narlikar, G.J., Sundaramoorthy, R., and Owen-Hughes, T. (2013) *Cell* 154, 490–503. | Patel, D.J. and Wang, Z. (2013) *Annu. Rev. Biochem.* 82, 81–118. | Serrano, L., Vazquez, B.N., and Tischfield, J. (2013) *Exp. Biol. Med.* 238, 259–270.

A Trusted Research Partner

Cell Signaling Technology (CST) strives to be your research partner for the study of epigenetics. As scientists, we understand the importance of using antibodies that work consistently each and every time. Our highly specific antibodies are directed against the most relevant targets in epigenetics and are painstakingly validated in relevant applications so you can feel confident in your results. In addition, we provide siRNAs, chemical modulators, and kits—all validated using the same rigorous quality standards—giving you the tools you need for every step of the experimental process. We are also here to help. Optimal antibody dilutions and recommended buffers are predetermined for you, saving you the time and trouble of additional optimization steps. Protocols and troubleshooting guides for commonly used applications are available on our website to ensure you get the expected results in the shortest amount of time. If you experience a problem in the lab, the same expert scientists who produced and validated your antibody or assay kit will respond to your email or phone call and help you, sharing their bench experience and data from their notebooks. We do all this because that's what we'd want if we were in the lab—because, actually, we are.

Research tools
for the study of epigenetics

CST has antibodies, kits, and reagents for each stage of the experimental process.

Primary Antibodies Over 400 primary antibodies directed against more than 230 protein targets. The collection is continually expanding, so please check our website frequently for a complete, up-to-date product list.

PTMScan® Kits and Services PTMScan Kits and Services utilize motif antibodies and LC-MS/MS technology to generate quantitative profiles of hundreds to thousands of proteins containing a particular type of post-translation modification.

Antibody Sampler Kits These kits allow for the simultaneous analysis of multiple nodes in a pathway of interest or modification sites within a protein of interest.

ELISA Kits and Antibody Arrays PathScan® ELISA Kits enable you to scale up your analysis to a 96-well format (384-well plates are also available on a custom basis), while antibody arrays allow you to monitor multiple pathway nodes in parallel using sandwich assays in a slide-based array.

SignalSilence® siRNA Rigorously validated siRNAs can be used to selectively knockdown a protein of interest.

Experimental Controls Control cell extracts, control proteins, blocking peptides, and isotype controls are available to help you verify antibody specificity.

Companion Products Secondary antibodies, loading controls, buffers, dyes, chemical modulators, detection reagents, protease inhibitors, proteases, and peptide standards are available to support your protocol.

Custom Products Our customs department will work with you if you require a product in a specific size or formulation, for your particular assay platform, or if you need a product validated using a specific measure or assay.

Tools for Chromatin Immunoprecipitation (ChIP)

Chromatin IP Kits **SimpleChIP® and SimpleChIP Plus Enzymatic Chromatin IP Kits** contain all the reagents needed to perform successful ChIP assays in cultured cells or in cultured cells and tissue samples, respectively.

Primary Antibodies **ChIP validated Primary Antibodies** have undergone in-house validation testing by CST scientists and are recommended for use in ChIP assays. Over 200 ChIP validated antibodies are currently available.

Control PCR Primers **SimpleChIP Control PCR Primers** contain a mix of two primers designed to amplify specific genomic loci. They can be used to amplify positive control sequences or used as a negative control to demonstrate antibody specificity.

Companion Products **SimpleChIP Companion Products** include protein G magnetic and agarose beads, isotype controls and magnetic separation racks. These products have been tested to work optimally with the SimpleChIP protocol.

The SimpleChIP Kit Advantage

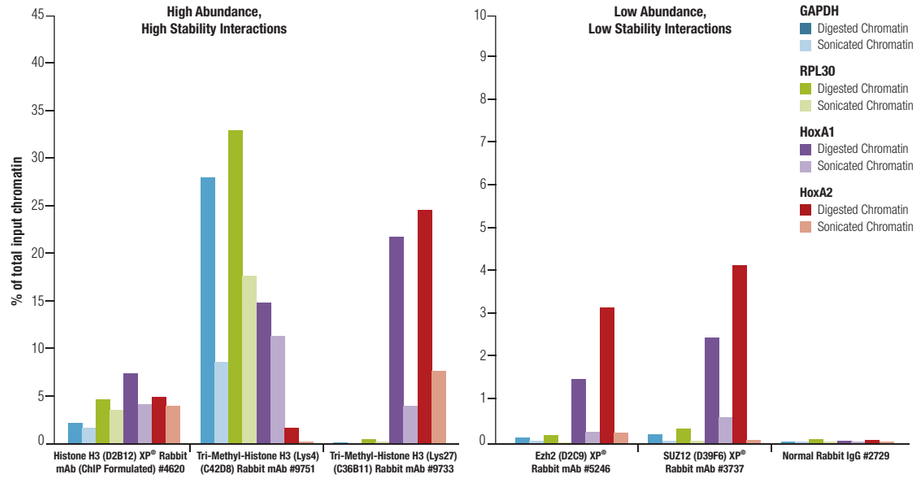
Detect Low Abundance Interactions with Enzyme Digestion

While effective, sonication is difficult to control and requires exposing the chromatin to harsh, denaturing conditions (i.e., high heat and detergent) that can damage both antibody epitopes and the genomic DNA. Enzymatic digestion, in contrast, uses micrococcal nuclease to gently fragment the chromatin into uniform pieces that are more conducive to immunoprecipitation.

Visit **www.cellsignal.com/chip** for a complete product list, additional data sets, protocols, and a troubleshooting guide.

Enzyme-digested chromatin is more conducive to immunoprecipitation than is sonicated chromatin. Chromatin prepared using this method consistently produces a stronger, more reliable signal, which is especially important if you're investigating low abundance, low stability interactions like the interaction between a polycomb group protein and a specific gene [e.g., Ezh2 or SUZ12, as illustrated in the figure on the right].

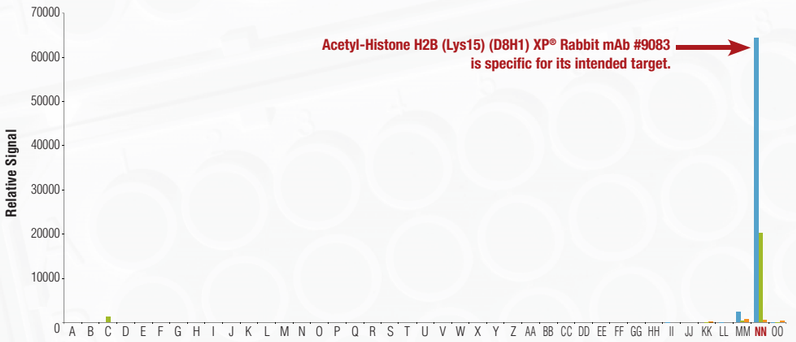
Enzyme-based Chromatin Digestion vs. Sonication-based Chromatin Fragmentation



Histone Modification-specific Antibodies

Peptide array assay confirms specificity of antibodies to defined histone modification sites.

Our modification-specific histone antibodies are validated with a peptide array assay similar to the one described by Fuchs, S.M., et al. [*Curr. Biol.* (2011) 21, 53–58]. These arrays assess antibody cross-reactivity against known modifications across all histone proteins in a single experiment. This method has the additional benefit of testing the effects of neighboring modifications on the ability of the antibody to detect a single modification site.



Acetyl-Histone H2B (Lys15) (D8H1) XP® Rabbit mAb #9083 displayed exceptional specificity for the H2B Lys15 modification site with minimal cross-reactivity for other modification sites. Quantification of peptide array analysis for #9083. Peptides specific for known modification sites were spotted in duplicate on the array at multiple concentrations and are indicated in blue, green, and orange. Arrays were probed using 0.1 µg/ml of the test antibody.

A	H3 (Lys4)	**H**	H3 (Lys23) Acetyl	**O**	H3 (Lys79)	**T**	H3 (Arg17) Asymmetric-di-methyl/(Lys18) Acetyl	**X**	H3 (Arg26) Asymmetric-di-methyl/(Lys27) Acetyl	**CC**	H4 (Lys12) Acetyl	**II**	H2A	**MM**	H2B (Lys12) Acetyl
B	H3 (Lys4) Acetyl	**I**	H3 (Lys27)	**P**	H3 (Lys79) Acetyl	**U**	H3 (Arg8) Symmetric-di-methyl/(Lys9) Acetyl	**Y**	H3 (Lys27) Acetyl /(Ser28) Phospho	**DD**	H4 (Lys16) Acetyl	**JJ**	H2A (Lys5) Acetyl	**NN**	**H2B (Lys15) Acetyl**
C	H3 (Lys9/Lys14/Lys18)	**J**	H3 (Lys27) Acetyl	**Q**	H3 (Thr3) Phospho /(Lys4) Acetyl	**V**	H3 (Lys9) Acetyl /(Ser10) Phospho	**Z**	H4 (Lys5/Lys8 /Lys12/Lys16)	**EE**	H4 (Lys20)	**KK**	H2B (Lys5/Lys12 /Lys15/Lys20)	**OO**	H2B (Lys20) Acetyl
D	H3 (Lys9) Acetyl	**K**	H3 (Lys36)	**R**	H3 (Arg2) Symmetric-di-methyl/(Lys4) Acetyl	**W**	H3 (Lys9) Acetyl /(Ser10/Thr11) Phospho	**AA**	H4 (Lys5) Acetyl	**FF**	H4 (Lys20) Acetyl	**LL**	H2B (Lys5) Acetyl		
E	H3 (Lys14) Acetyl	**L**	H3 (Lys36) Acetyl	**S**	H3 (Arg2) Asymmetric-di-methyl/(Lys4) Acetyl			**BB**	H4 (Lys8) Acetyl	**GG**	H4 (Lys91)				
F	H3 (Lys18) Acetyl	**M**	H3 (Lys56)							**HH**	H4 (Lys91) Acetyl				
G	H3 (Lys23)	**N**	H3 (Lys56) Acetyl												

Cell Signaling TECHNOLOGY®

We've got it covered

Our total and modification-specifc antibody portfolio covers critical targets within the epigenetic pathways.

- ACF1
- Mono-Methyl Arginine
- Asymmetric Di-Methyl Arginine
- Symmetric Di-Methyl Arginine
- ARID1A/BAF250A
- ASF1A
- ASF1B
- ASH2L
- Bmi1
- BORIS
- Brd2
- BRD4
- Brg1
- BRM
- BTAF1
- CABIN1
- CAS20
- CBP
- Acetyl-CBP (Lys1535)/p300 (Lys1499)
- CDK7
- CDK8
- CDK9
- CENP-A
- Phospho-CENP-A (Ser7)
- CHAF1A
- CHD1
- CHD1L
- CHD3
- CHD4
- CHD7
- CHD8
- CLOCK
- CtBP1
- CtBP2
- CTCF
- CTDSPL2
- Phospho-CTDSPL2 (Ser104)
- CTR9
- CXXC1
- Cyclin T1
- DBC1
- Phospho-DBC1 (Thr454)
- DMAP1
- DNMT1
- DNMT3A
- DNMT3B
- DNMT3L
- DR1
- EAF2
- ELP1/IKBKAP
- ELP3
- ESET
- EWS
- Ezh2
- FCP1
- G9a/EHMT2
- GCN5L2
- HELLS
- HEXIM1
- HIRA
- Histone Deacetylase 1 (HDAC1)
- Histone Deacetylase 2 (HDAC2)
- Histone Deacetylase 3 (HDAC3)
- Phospho-HDAC3 (Ser424)
- Histone Deacetylase 4 (HDAC4)
- Phospho-HDAC4 (Ser246)/HDAC5 (Ser259)/HDAC7 (Ser155)
- Phospho-HDAC4 (Ser632)/HDAC5 (Ser661)/HDAC7 (Ser486)
- Histone Deacetylase 6 (HDAC6)
- Histone H2A
- Acetyl-Histone H2A (Lys5)
- Ubiquityl-Histone H2A (Lys119)
- Histone H2A.X
- Phospho-Histone H2A.X (Ser139)
- Phospho-Histone H2A.X (Ser139/Tyr142)
- Histone H2A.Z
- Histone H2B
- Acetyl-Histone H2B: Lys5, Lys12, Lys15, Lys20
- Phospho-Histone H2B (Ser14)
- Ubiquityl-Histone H2B (Lys120)
- Histone H3
- Acetyl-Histone H3: Lys9, Lys9/Lys14, Lys14, Lys18, Lys23, Lys27, Lys36, Lys56
- Acetyl- and Phospho-Histone H3 (Lys9/Ser10)
- Methyl-Histone H3 (Arg2)
- Symmetric-di-methyl Histone H3 (Arg8)
- Mono-Methyl-Histone H3: Lys4, Lys9, Lys27, Lys36, Lys79
- Di-Methyl-Histone H3: Lys4, Lys9, Lys27, Lys36, Lys79
- Di-/Tri-Methyl-Histone H3 (Lys9)
- Tri-Methyl-Histone H3: Lys4, Lys9, Lys27, Lys36, Lys79
- Pan-Methyl-Histone H3 (Lys9)
- Phospho-Histone H3: Ser10, Ser28, Thr3, Thr11, Thr22
- Cleaved Histone H3 (Thr22)
- Histone H4
- Acetyl-Histone H4: Lys5, Lys8, Lys12, Lys16
- Mono-Methyl-Histone H4 (Lys20)
- Di-Methyl-Histone H4 (Lys20)
- Tri-Methyl-Histone H4 (Lys20)
- HMGA1
- HMGA2
- HMGB1
- HMGB2
- HMGN1
- HMGN2
- hnRNP A2/B1
- HP1α
- HP1β
- HP1γ
- Phospho-HP1γ (Ser83)
- JARID1: A, B, C
- Jarid2
- JMJD1A, B
- JMJD2: A, B, C
- JMJD3
- LSD1
- Acetylated-Lysine
- MacroH2A1
- MacroH2A1.1
- MacroH2A1.2
- MBD3
- MeCP2
- MED12
- MED26
- Menin
- MEP50
- MLLT1/ENL
- MORF4L1/MRG15
- MTA1
- NCoR1
- Nucleolin
- Nucleomethylin
- NUT
- PAF1
- PCAF
- PHC1
- PHF2
- PHF20
- POLR3A
- Pontin/RUVBL1
- PRMT1
- PRMT4/CARM1
- PRMT5/Skb1Hs Methyltransferase
- RAD21
- RBAP46
- RBAP46/RBAP48
- RBBP5
- Reptin/RuvBL2
- Ring1A
- RING1B
- RNF20
- RNF40
- Phospho-Rpb1 CTD: Ser2, Ser2/Ser5, Ser5, Ser7
- RSF1
- SATB1
- Phospho-SATB1 (Ser47)
- SET7/SET9
- SET8
- SIN3A
- SirT1
- Phospho-SirT1: Ser27, Ser47
- SirT2
- SirT3
- SirT5
- SirT6
- SirT7
- SMARCA1
- SMARCAD1
- SMARCC1/BAF155
- SMARCC2/BAF170
- SMYD2
- SMYD3
- SNF2H
- SNF5
- SP1
- SPT4
- SPT5
- SPT16
- SRC-1
- SRC-3
- Phospho-SRC-3 (Thr24)
- SSRP1
- SSU72
- SUV39H1
- SUZ12
- TAF1
- TAF15
- TBP
- TCEB3/Elongin A
- TFII-I
- TFIIB
- TFIIE-α
- TFIIF-α
- TH1L
- Tip60
- Topoisomerase IIα
- Phospho-Topoisomerase IIα: Ser1106, Ser1469
- TRIM29/ATDC
- TRRAP
- UHRF1
- WDR5
- WSTF
- XPB
- XPD
- YY1

Motif and PTM-specific Antibodies

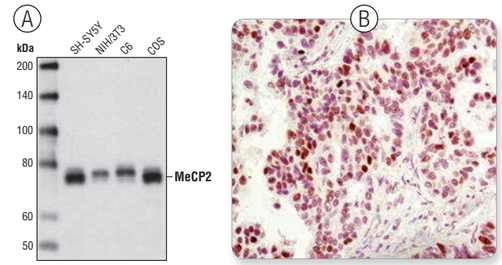
Motif and PTM-specific antibodies can be used to generate quantitative profiles of specific motifs or modification phosphorylation sites of cellular proteins, respectively. Modifications that can be measured include methylation and acetylation.

For a complete listing of our Motif and PTM-Specific Antibodies: **www.cellsignal.com/PTMabs**

Tools to Support Your Epigenetics Workflow

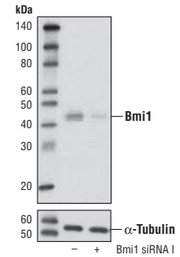
Antibodies to assess localization of key epigenetics targets

MeCP2 (D4F3) XP® Rabbit mAb #3456: WB analysis of extracts from various cell lines **(A)** using #3456. IHC analysis of paraffin-embedded human lung carcinoma **(B)** using #3456.



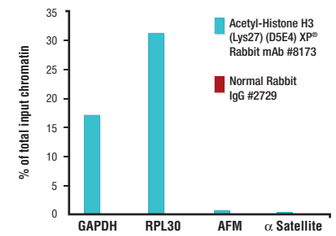
siRNAs to confirm target specificity

SignalSilence® Bmi1 siRNA I #6442: WB analysis of extracts from HeLa cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-) or #6442 (+), using Bmi1 (D20B7) XP® Rabbit mAb #6964 (upper) or α-Tubulin (11H10) Rabbit mAb #2125 (lower). The Bmi1 (D20B7) XP® Rabbit mAb confirms silencing of Bmi1 expression, while the α-Tubulin (11H10) Rabbit mAb is used as a loading control.



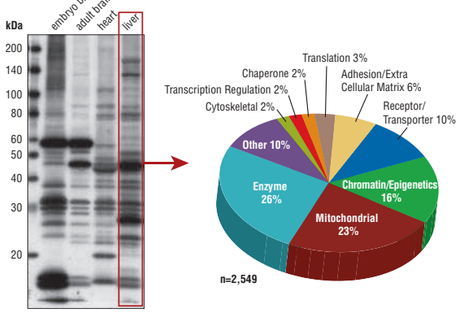
ChIP validated kits, primers and antibodies to examine protein-DNA interactions

Acetyl-Histone H3 (Lys27) (D5E4) XP® Rabbit mAb #8173: Chromatin IPs were performed with cross-linked chromatin from 4 x 10^6 HeLa cells and either 5 µl of #8173 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 a percent of total input chromatin.



PTMScan Kits and Services for PTM profiling

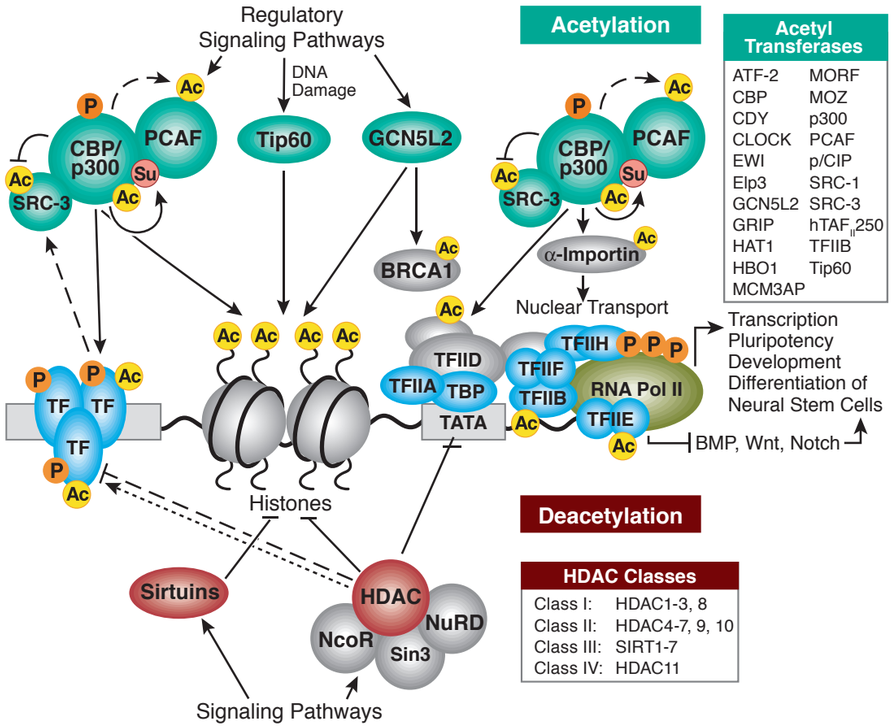
Acetylated-Lysine (Ac-K²-100) Rabbit mAb #9814: WB analysis of extracts from various mouse tissues using Acetylated-Lysine (Ac-K²-100) Rabbit mAb shows differences in acetylation among tissue types. Specific proteins can be identified using AcetylScan® Kits and Services.



Cell Signaling TECHNOLOGY®

Histone Acetylation

A wide range of tools helps you examine all aspects of epigenetics.

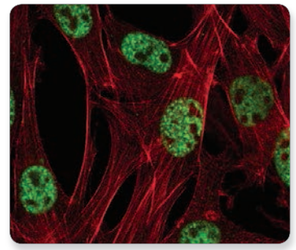


Lysine acetylation is a reversible post-translational modification that plays a crucial role in regulating protein function, chromatin structure, and gene expression. Many transcriptional coactivators possess intrinsic acetylase activity, while transcriptional corepressors are associated with deacetylase 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.

Selected Reviews: Albaugh, B.N., Arnold, K.M., and Denu, J.M. (2011) *Chem. Bio. Chem.* 12, 290–298. | Choudhary, C., Kumar, C., Gnad, F., Nielsen, M.L., Rehman, M., Walther, T.C., Olsen, J.V., and Mann, M. (2009) *Science* 325, 834–840. | Dali-Youcef, N., Lagouge, M., Froelich, S., Koehl, C., Schoonjans, K., and Auwerx, J. (2007) *Ann. Med.* 39, 335–345. | Finkel, T., Deng, C.H., and Mostoslavsky, R. (2009) *Nature* 460, 587–591. | Haberland, M., Montgomery, R.L., and Olson, E.N. (2009) *Nat. Rev. Genet.* 10, 32–42. | Peng, L. and Seto, E. (2011) *Handbook Exp. Pharmac.* 206, 39–56. | Spange, S., Wagner, T., Heinzel, T., and Krämer, O.H. (2009) *Int. J. Biochem. Cell Biol.* 41, 185–198. | Yang, X.J. and Seto, E. (2007) *Oncogene* 26, 5310–5318. | Yang, X.J. and Seto, E. (2008) *Mol. Cell* 31, 449–461.

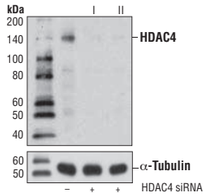
Antibodies to assess key cellular target localization

CBP (D6C5) Rabbit mAb #7389: Confocal IF analysis of HeLa cells using #7389 (green). Actin filaments were labeled with DY-554 phalloidin (red).



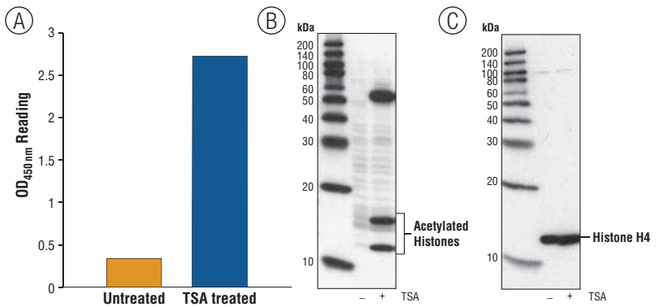
SignalSilence siRNA for knockdown studies

SignalSilence® HDAC4 siRNA I #7595: WB analysis of extracts from HeLa cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), #7595. (+), or SignalSilence® HDAC4 siRNA II #7609 (+), using HDAC4 (D15C3) Rabbit mAb #7628 (upper) or α-Tubulin (11H10) Rabbit mAb #2125 (lower). The HDAC4 (D15C3) Rabbit mAb confirms silencing of HDAC4 expression, while the α-Tubulin (11H10) Rabbit mAb is used as a loading control.



PathScan ELISA Kits for quantitative analysis

PathScan® Acetyl-Histone H4 Sandwich ELISA Kit #7238: Treatment of Jurkat cells with TSA causes accumulation of acetylation on Histone H4, detected by #7238, but does not affect the level of total Histone H4 protein, detected by Western analysis. $OD_{450\,nm}$ readings are shown **(A)**, with the corresponding WB using the Acetylated Lysine Mouse mAb (Ac-K-103) #9681 **(B)** or Histone H4 Antibody #2592 **(C)**.



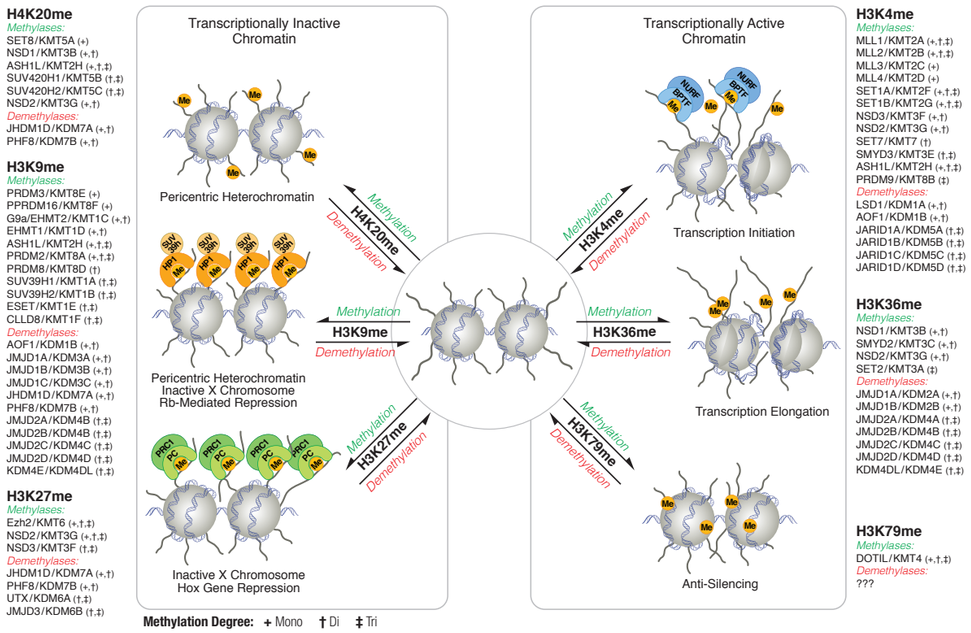
Acetylation Proteomics

The AcetylScan® Kits and Services provide a unique strategy for global analysis of HDAC and HAT activity on protein acetylation. AcetylScan® products utilize antibodies with high afinity to acetylated-lysine (Ac-K) to enrich acetylated peptides from protease-digested cell or tissue samples. The samples are then analyzed by liquid chromatography (LC) tandem mass spectrometry (MS/MS) to generate quantitative profiles of acetylation sites in cellular proteins.

www.cellsignal.com/acetylation

Histone Methylation

Comprehensive pathway coverage simplifies your experimental design.



Lysine methylation has been implicated in both transcriptional activation (H3K4, K36, K79) and repression (H3K9, K27, H4K20); the outcome depending on both the degree and localization of the specific methyl mark. Lysines can have three different methylation states (mono-, di- and tri-) that are associated with different nuclear features and transcriptional states. In order to establish these methylation states, cells have enzymes that add (lysine methyltransferases-KMTs) and remove (lysine demethylases-KDMs) different degrees of methylation from specific lysines within the histones.

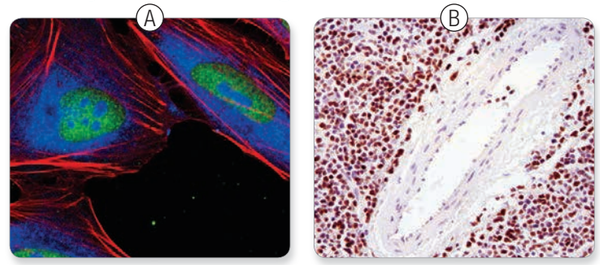
Arginines can be mono-methylated, and symmetrically or asymmetrically di-methylated by a family of protein arginine methyl transferases (PRMTs). There are three types of PRMTs, which are classified by their ability to generate the different methylation states. All three types of PRMTs can mono-methylate arginines. The mono-methylated arginines are further methylated by type I PRMTs to generate asymmetric di-methyl arginines, or by type II PRMTs to form symmetric-dimethyl ariginines. Type III PRMTs are only able to mono-methylate the arginine residues. Much like lysines, both the degree and localization of arginine methylation influence transcriptional outcome.

Since the methyl group is uncharged and chemically inert, the impact these modifications have is through recognition and recruitment of chromatin modifying enzymes containing methyl-lysine or methyl-arginine binding domains. Chromodomains, PHD fingers, PWWP domains and WD-40 domains are among a growing list of methyl-lysine binding modules, while Tudor domains can bind either methyl-lysine or methyl-arginine marks. Lysine and arginine methylation provides a binding surface for these enzymes, which then regulate chromatin condensation, nucleosome mobility, active and inactive transcription, as well as DNA repair and replication. In addition, methylation can block binding of proteins that interact with unmethylated histones or directly inhibit catalysis of other regulatory modifications on neighboring residues.

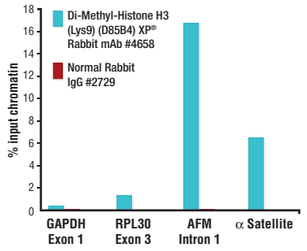
Selected Reviews: Kooistra, S,M. and Helin, K. (2012) *Nat. Rev. Mol. Cell Biol.* 13, 297–311. | Tee, W.W. and Reinberg, D. (2014) *Development* 141, 2376–2390. | Greer, E.L. and Shi, Y. (2012) *Nat. Rev. Genet.* 13, 343–357. | Herz, H.M., Garruss,A., and Shilatifard, A. (2013) *Trends Biochem. Sci.* 38, 621–639. | Black, J.C., Van Rechem, C., and Whetstine, J.R. (2014) *Mol. Cell.* 48, 491–507. | Van Rechem, C. and Whetstine, J.R. (2014) *Biophys. Acta.* Epub ahead of print. | Yang, Y. and Bedford, M.T. (2013) *Nat. Rev. Cancer* 13, 37–50. | Gayatri, S. and Bedford, M.T. (2014) *Biochim. Biophys. Acta.* 839, 702–710. | Molina-Serrano, D., Schiza, V., and Kirmizis, A. (2013) *Biochem. Soc. Trans.* 41, 751–759.

Antibodies to assess key cellular target localization

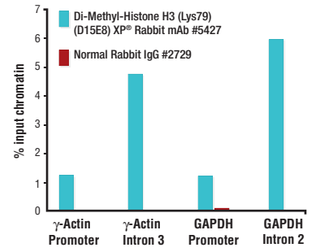
Ezh2 (D2C9) XP® Rabbit mAb #5246: Confocal IF analysis of HeLa cells **(A)** using #5246 (green) and S6 Ribosomal Protein (54D2) Mouse mAb #2317 (blue). Actin filaments were labeled with DY-554 phalloidin (red). IHC analysis of paraffin-embedded human lymphoma **(B)** using #5246.



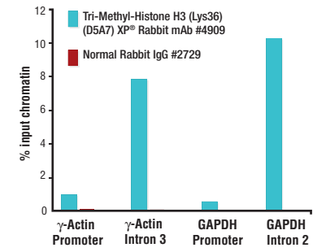
SimpleChIP Kits, Primers, and Antibodies for quantitative analysis



Di-Methyl-Histone H3 (Lys9) (D85B4) XP® Rabbit mAb #4658: Chromatin IPs were performed with cross-linked chromatin from 4 x 10^6 HeLa cells and either 20 µl of #4658 or 2 µl of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003 and the following primers: #5516, #7014, #5098, #4486



Di-Methyl-Histone H3 (Lys79) (D15E8) XP® Rabbit mAb #5427: Chromatin IPs 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 and the following primers: #5037, #5047, #4471, #4478

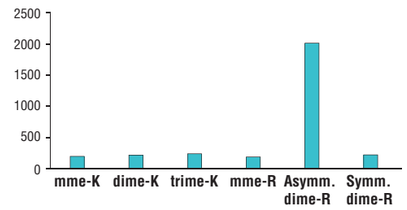


Tri-Methyl-Histone H3 (Lys36) (D5A7) XP® Rabbit mAb #4909: Chromatin IPs were performed with cross-linked chromatin from 4 x 10^6 HeLa cells and either 10 µl of #4909 or 2 µl of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003 and the following primers: #5037, #5047, #4471, #4478

PRIMERS

SimpleChIP® Human GAPDH Exon 1 Primers #5516	SimpleChIP® Human α Satellite Repeat Primers #4486	SimpleChIP® Human GAPDH Promoter Primers #4471
SimpleChIP® Human RPL30 Exon 3 Primers #7014	SimpleChIP® Human γ-Actin Promoter Primers #5037	SimpleChIP® Human GAPDH Intron 2 Primers #4478
SimpleChIP® Human AFM Intron 1 Primers #5098	SimpleChIP® Human γ-Actin Intron 3 Primers #5047	

Kits and Services for PTM Profiling



Asymmetric Di-Methyl Arginine Motif [adme-R] Rabbit mAb #13522: The specificity of #13522 was determined using peptide ELISA. The figure demonstrates that the antibody is specific for asymmetric di-methyl arginine and does not react with mono-methyl, di-methyl or tri-methyl lysine and does not react with mono-methyl or symmetric di-methyl arginine.

Methylation Proteomics

The MethylScan® Kits and Services employ a proprietary methodology that allows for global analysis of methyltransferase and demethylase activity on protein methylation. Our methodology uses antibodies with high affinity to mono-methylated arginine, symmetric and asymmetric di-methyl arginine, and mono-methylated lysine to enrich methylated peptides from protease digested cell or tissue samples. The samples are then analyzed by liquid chromatography (LC) tandem mass spectrometry (MS/MS) to generate quantitative profiles of methylation sites in cellular proteins.

www.cellsignal.com/methylation

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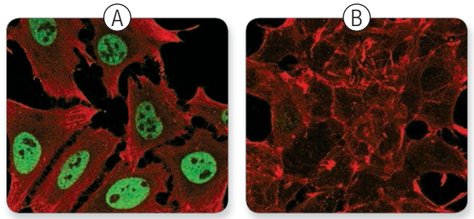
ATP-dependent Remodeling Proteins

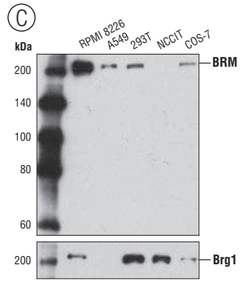
ATP-dependent remodeling proteins make structural changes to chromatin by using their ATPase catalytic subunit to disrupt histone-DNA contacts and reposition nucleosomes, exposing regions of DNA to the regulatory proteins necessary for transcription, DNA replication, and repair.

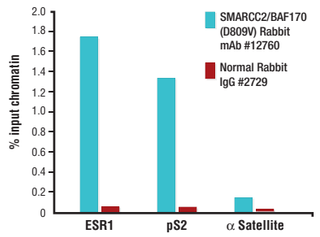
SWI/SNF Complex

The SWI/SNF complex (BAF and PBAF complexes in mammals) consists of multiple subunits and contains either a BRM (SMARCA2) or a BRG1 (SMARCA4) protein that acts as an ATPase. Components of the SWI/SNF complex are commonly mutated in cancer and are the focus of many research efforts as potential therapeutic targets.



BRM (D9E8B) XP® Rabbit mAb #11966: Confocal IF analysis of HeLa (positive, **A**) and NCCIT (negative, **B**) cells using #11966 (green) and β-Actin (8H10D10) Mouse mAb #3700 (red). WB analysis of extracts from various cell lines **(C)** using #11966 or Brg1 (A52) Antibody #3508.

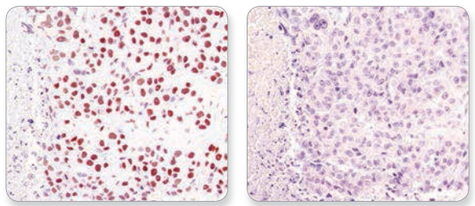




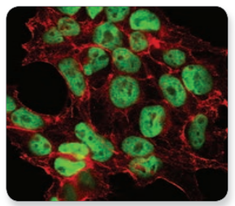
SMARCC2/BAF170 (D8O9V) Rabbit mAb #12760: Chromatin IPs were were performed with cross-linked chromatin from 4 x 10^6 MCF7 cells, grown in phenol red-free medium and 5% charcoal-stripped FBS for 4 d followed by treatment with β-estradiol (10 nM, 45 min), and either 10 µl of #12760 or 2 µl of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human ESR1 Promoter Primers #9673, SimpleChIP® Human pS2 Promoter Primers #9702, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as a percentage of the total input chromatin.

NuRD Complex

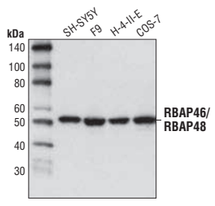
The transcriptional repressor nucleosome remodeling and histone deacetylase (NuRD) complex is composed of multiple subunits, including histone deacetylases (HDAC1 and HDAC2) and the ATPase (CHD3, CHD4, and CHD5). The NuRD complex plays an important role in regulating genes responsible for embryonic stem cell pluripotency and differentiation.



MTA1 (D40D1) XP® Rabbit mAb #5647: IHC analysis of paraffin-embedded human lung carcinoma using #5647 in the presence of control peptide (left) or antigen-specific peptide (right).



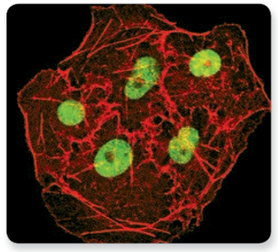
CHD4 (D4B7) Rabbit mAb #12011: Confocal IF analysis of 293T cells using #12011 (green). Actin filaments were labeled with DY-554 phalloidin (red).



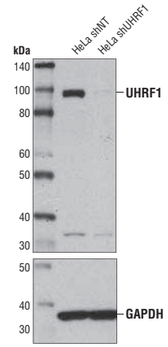
RBAP46/RBAP48 (D4F8) Rabbit mAb #9067: WB analysis of extracts from various cell lines using #9067.

DNA Methylation

DNA methylation is one of the most studied epigenetic modifications. Methylation at cytosine residues results in gene silencing and is critical for proper regulation of gene expression, genomic imprinting, and development. Improper DNA methylation, including hypermethylation of CpG islands in the promoter region of key genes, has been found to be associated with cancer.



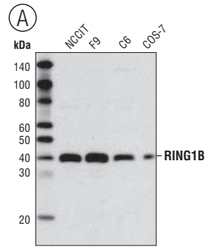
DNMT1 (D63A6) XP® Rabbit mAb #5032: Confocal IF analysis of COS-7 cells using #5032 (green). Actin filaments were labeled using DY-554 phalloidin (red).

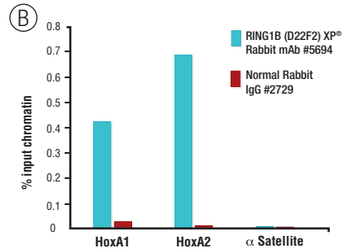


UHRF1 (D6G8E) Rabbit mAb #12387: WB analysis of extracts from HeLa cells, expressing either nontargeting shRNA (HeLa shNT) or shRNA targeting UHRF1 (HeLa shUHRF1), using #12387 (upper) or GAPDH (D16H11) XP® Rabbit mAb #5174 (lower).

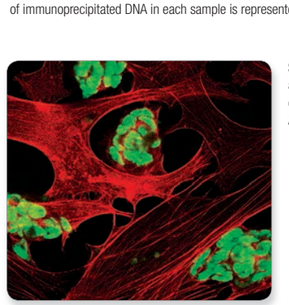
Polycomb Group Proteins

Polycomb group (PcG) proteins help maintain cell identity, stem cell self-renewal, cell cycle regulation, and oncogenesis by silencing gene that promote cell lineage specification, cell death, and cell cycle arrest. PcG proteins exist in two complexes: PRC2 (EED-EZH2), which methylates histone H3 on Lys27 (H3K27), and the PRC1 complex, which ubiquitinylates histone H2A on Lys119 in response to H3K27 methylation.





RING1B (D22F2) XP® Rabbit mAb #5694: WB analysis of extracts from various cell lines **(A)** using #5694. Chromatin IPs were performed with cross-linked chromatin from 4 x 10^6 NCCIT cells **(B)** and either 10 µl of #5694 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 HoxA1 Intron 1 Primers #7707, SimpleChIP® Human HoxA2 Promoter Primers #5517, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as a percentage of the total input chromatin.



SUZ12 (D39F6) XP® Rabbit mAb #3737: Confocal IF analysis of mouse embryonic stem cells growing on mouse embryonic fibroblast (MEF) feeder cells using #3737 (green). Actin filaments were labeled with DY-554 phalloidin (red).

Disease Connection

A common feature of cancer cells is a reversal in the normal bimodal genomic methylation pattern – more common, in fact, than actual gene mutations. This observation has led investigators to identify numerous tumor suppressor genes based on aberrations in the methylation pattern of their promoters. *MGMT*, for example, is a DNA repair gene that has been found to be epigenetically silenced in cancer. Silencing of this gene can cause genomic instability and lead an early-stage tumor cell to acquire additional oncogenic mutations in genes like *TP53* or *K-Ras*. This finding suggests that epigenetic silencing of key genes can affect the pathological progression of a tumor at multiple stages. Moreover, it suggests that methylation patterns may provide good biomarkers for early cancer diagnostics, and that proteins responsible for maintaining epigenetic marks may make good targets for cancer therapeutics. Investigators are using data from both genomic and epigenomic research efforts to ensure that these possibilities become clinical reality.

Selected Reviews:

Tsai, H.C., and Baylin, S.B. (2011) *Cell Res.* 21, 502–517.

Lopez, J., Perchard, M., and Coley, H.M., et al. (2009) *Brit. J. Cancer* 100, 571–577.

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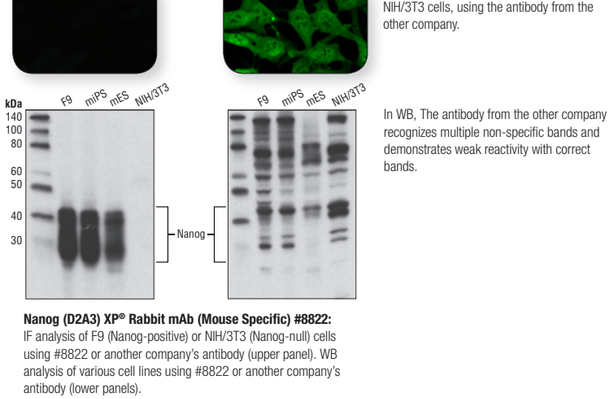
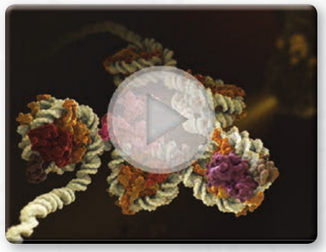


Nanog (D2A3) XP® Rabbit mAb (Mouse Specific) #8822: IF analysis of F9 (Nanog-positive) or NIH/3T3 (Nanog-null) cells using #8822 or another company's antibody (upper panel). WB analysis of various cell lines using #8822 or another company's antibody (lower panels).



An Introduction to Epigenetics

Please visit **www.cellsignal.com/epivideo** to view this 3D rendered animation containing an introduction to the nucleosome, histone code, and euchromatin and heterochromatin states.

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At CST, providing exceptional customer service and technical support are top priorities. Our scientists work at the bench daily to produce and validate our antibodies, so they have hands-on experience and in-depth knowledge of each antibody's performance. In the process, these same scientists generate valuable reference information that they use to answer your questions and help troubleshoot your experiment by phone or email.

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CST Product Scientists: Troy, PhD (left) has been with CST since 2010 and **Christina** (right) has been with CST since 2007.

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FRONT COVER IMAGE:
Methylation of cytosine bases in regions called CpG islands is a hallmark of transcriptionally repressed heterochromatin. These methylated cytosines in turn recruit proteins like methyl-CPG binding protein 2 (MeCP2; gray) and heterochromatin protein 1 (HP1; orange). These proteins are thought to maintain a repressive state of chromatin by inducing histone deacetylation by HDACs (purple) as well as histone tail methylation by histone methyltransferase enzymes (red).

Sandra, Associate Scientist has been with CST since 1996.

FOUNDED BY RESEARCH SCIENTISTS IN 1999, Cell Signaling Technology (CST) is a private, family-owned company with over 400 employees worldwide. Active in the field of applied systems biology research, particularly as it relates to cancer, CST understands the importance of using antibodies with high levels of specificity and lot-to-lot consistency. It's why we produce all of our antibodies in house, and perform painstaking validations for multiple applications. And the same CST scientists who produce our antibodies also provide technical support for customers, helping them design experiments, troubleshoot, and achieve reliable results. We do this because that's what we'd want if we were in the lab. Because, actually, we are.

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