Antibodies, Kits, and Reagents for 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:

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.

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.

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. [J. 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.

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 sites. They can be used to amplify positive control sequences or used as a negative control to demonstrate antibody specificity.

Tools Overview

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

Visit www.cellsignal.com/epilearnmore for a complete product list, additional data sets, protocols, and a troubleshooting guide.
We’ve got it covered

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

Motif and PTM-specific Antibodies

Mott and PTM-specific antibodies can be used to generate quantitative profiles of specific motifs or modifications 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

MCCP2 (24F1) 3F Rabbit mAb #4616: IIS analysis of extracts from various cell lines (A) using PTMScan® analysis of parallel anti-embossed Human lung cancer samples (B) using #4616.

siRNAs to confirm target specificity

SignalSilence® Bmi1 siRNA (H-14642) IIS analysis of extracts from HA-A cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (–) or #6442 (+), using Bmi1 (D20B7) XP® Rabbit mAb #8173 (lower). The Bmi1 (D20B7) XP® Rabbit mAb confirms absence of Bmi1 expression, while the c-Myc (11H10) Rabbit mAb is used as a loading control.

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

Acetyl-Histone H3 (Lys9/14/18) (20E6) SP® Rabbit mAb #6173: Chromatin Pte were performed with cross-linked chromatin from 4 × 10⁷ MCF-7 cells and with either 1:20 of 4% H2O2 or 2% formaldehyde. Cross-linked chromatin was prepared using ChIP-Grade Cross-Linking Fixation Kit (Magnetic Beads) #6202. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human SATIP from 1 Primers #5725, SimpleChIP® Human KIF22 Exon 3 Primers #5729, SimpleChIP® Human KIF22 Exon 3 Primers #5714, SimpleChIP® Human KIF22 Exon 3 Primers and SimpleChIP® Human KIF22 Exon 3 Primers. The amount of immunoprecipitated DNA in each sample is represented as a percent of total input chromatin.

PTMScan Kits and Services for PTM profiling

Acetyl-Lysine (Ac-K 4–10) Rabbit mAb #4524: IIS analysis of extracts from various human tissues using Acetyl-Lysine (Ac-K 4–10) Rabbit mAb shows differences in acetylated lysines across tissues. Specific proteins can be identified using Acetyl-Lysine® Kits and Services.
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) 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:

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 affinity to acetylated lysine (Ac-K) to enrich acetylated peptides from protease-digested cell or tissue samples. The samples are then analyzed by liquid chromatography - tandem mass spectrometry (LC-MS/MS) to generate quantitative profiles of acetylation sites in cellular proteins.

www.cellsignal.com/acetylation
Lysine methylation has been implicated in both transcriptional activation (H3K4, K36, K79) and repression (H3K27, 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 lysyl-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 II PRMTs to form symmetric dimethyl arginines. 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 unmodified histones or directly inhibit catalysis of other regulatory modifications on neighboring residues.


Methylation Proteomics

The MethyScribe® Kits and Services employ a proprietary methodology that allows for global analysis of methylation and demethylation 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
Chromatin Dynamics

Validated antibodies and reagents move your research forward faster.

ATP-dependent Remodeling Proteins

ATP-dependent remodeling proteins make structural changes to chromatin by using their ATPase catalytic subunits 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.

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.

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.

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.

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:

www.cellsignal.com/epilearnmore
A trusted partner at the bench

We validate each antibody in-house, using appropriate methods to verify specificity, sensitivity, and reproducibility, so you can be confident in your experimental results.

Does your antibody meet your expectations?

WE DO THE RELEVANT VALIDATION, SO YOU DON’T HAVE TO...

- Appropriate signal observed in all recommended applications
- Clean band at appropriate molecular weight observed by western blot
- Specificity confirmed by one or more of the following:
  - Appropriate subcellular localization
  - Overexpression
  - Activator or inhibitor treatment
  - Positive and negative cell lines or tissues
  - Phosphatase treatment
  - RNA interference
  - Peptide ELISA or array
- Specific reactivity confirmed in multiple biologically relevant species and cell lines
- Lot-to-lot consistency, calibrated for reliable results
- Proven protocols for results you can reproduce

Are you confident that your antibody is specific?

WB and IF analysis show that the other company’s antibody lacks specificity

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.

CST Technical Support

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, those same scientists generate valuable reference information that they use to answer your questions and help troubleshoot your experiment by phone or email.

www.cellsignal.com/support (USA & Europe)
www.cst-c.com.cn/support (China)
www.cstj.co.jp/support (Japan)
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).