EMD MILLIPORE

Product Selection Guide

Shaping Epigenetics Discovery

Chromatin, DNA methylation, RNA analysis

EMD Millipore is a division of Merck KGaA, Darmstadt, Germany

Platforms, Technologies, and Services

EMD Millipore is committed to the advancement of life science research and therapeutic development. We've developed epigenetics technology that is more accessible and easier to use than ever, enabling you to design the most ambitious experiments to interrogate gene regulation.

With our legacy of expertise from Chemicon® and Upstate®, we are committed to molding sophisticated yet simple solutions for understanding epigenetic regulation.

ANTIBODIES AND IMMUNOASSAYS

EMD Millipore offers an extensive, focused portfolio of antibodies and immunoassays including validated antibodies for effective immunoprecipitation of chromatin, RNA-binding proteins, modified histones, and transcriptional proteins. EMD Millipore's antibodies are developed in-house, tested in multiple applications for specificity, and quality controlled where necessary to ensure optimal performance in your experiment.

CHROMATIN ANALYSIS

Count on EMD Millipore for advanced technologies for effective chromatin analysis, from chromatin assembly to endpoint assays. EMD Millipore offers a growing selection of kits, antibodies, and assays, to support the complete workflow in almost every area of epigenetic research, from single-locus to genome-wide analyses, high-throughput platforms, effective magnetic-bead technology, proven controls, and a greater capacity for automation, all backed by expert technical support.

HISTONE MODIFICATIONS

Understanding histone modifications is key to uncovering epigenetic mechanisms of gene regulation. EMD Millipore grasps the complexity of this research and provides a growing line of kits, antibodies, and assays for studies of histone and histone-variant phosphorylation, methylation, acetylation, ubiquitination and citrullination--the most widely studied histone modifications in epigenetics today.

DNA METHYLATION

EMD Millipore continues to build upon the expertise of Upstate® and Chemicon® to develop effective tools for DNA methylation analysis. Our product line simplifies bisulfite modification and methylation-specific PCR of CpG islands, with faster protocols and effective antibodies against important markers such as 5-hydroxymethylcytosine, 5-methylcytosine, TET enzymes, and DNA methyltransferases.

TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL CONTROL

Confidently investigate mechanisms of transcriptional and post-transcriptional control with EMD Millipore's line of RNA-binding protein immunoprecipitation (RIP) kits containing validated antibodies and primer sets, and proven controls. Look to EMD Millipore for kits, antibodies, and assays for the analysis of proteins involved in mRNA stability, pre-mRNA processing, ncRNA-mediated gene regulation, mRNA transport and translational regulation.

CALBIOCHEM® SMALL MOLECULES

Small-molecule compounds, including inhibitors, activators, and other pathway modulators, are critical tools for researchers studying gene regulation. Chemical genetics, in which loss of function is imposed using small molecules, can reveal functions and interactions of proteins affecting gene expression through epigenetic or other mechanisms. EMD Millipore's Calbiochem® reagents have been cited in thousands of peer-reviewed publications. From libraries and pathway panels to individual reagents, the Calbiochem® line of products offers the widest and most cited selection of inhibitors and activators worldwide.

FLOW CYTOMETRY ASSAYS AND SYSTEMS

For epigenetic and gene regulation analysis at the cellular level, flow cytometry is ideal for simultaneously measuring multiple parameters on individual cells. Guava easyCyte™ flow cytometers provide direct, precise measurement via microcapillary technology that translates into smaller samples, less reagents, and minimal waste. FlowCellect™ kits and Milli-Mark™ conjugated antibodies are optimized for guava easyCyte™ systems and compatible with traditional core lab environments. Along with application-specific software modules, they provide a complete solution for flow cytometry.

MILLIPLEX® MAP MULTIPLEX ASSAYS

MILLIPLEX® MAP bead-based multiplex assays enable multianalyte elucidation of gene regulation networks using a small sample volume. Using Luminex® technology, the MILLIPLEX® platform enables the simultaneous detection of multiple soluble or intracellular biomarkers, including modified histones and absolute quantitation of phosphorylation. These flexible and customizable assays are exhaustively tested and validated for sensitivity, specificity, reproducibility and wide dynamic range.

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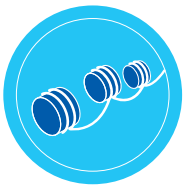




For a complete list of our epigenetics products, please visit: www.millipore.com/epigenetics

Introduction

Epigenetics describes heritable changes in gene expression caused by non-genetic mechanisms instead of by alterations in DNA sequence. These changes can be cell- or tissue-specific, and can be passed on to multiple generations. Epigenetic regulation enriches DNA-based information, allowing a cell to vary its response across diverse biological and environmental contexts. Although epigenetic mechanisms are primarily centered in the nucleus, these mechanisms can be induced by environmental signals such as hormones, nutrients, stress, and cellular damage, pointing to the involvement of cytoplasmic and extracellular factors in epigenetic regulation.

Epigenetic changes can effect transcriptional and post-transcriptional regulation via the following mechanisms:

- Histone modification
- Positioning of histone variants
- Chromatin and nucleosome remodeling
- DNA methylation
- Small and non-coding RNA-mediated epigenetic regulation

These mechanisms, in cooperation with transcription factors and other nucleic acid-binding proteins, regulate gene expression, resulting in cellular diversity although DNA sequences are virtually identical from cell to cell. Epigenetic mechanisms of gene regulation impacts diverse areas of research—from agriculture to human health.

Epigenetics research tools are used across a wide variety of research areas, including the following:

- Neuroscience
- Cancer
- Stem cells
- Cell differentiation
- Embryonic development
- Aging

As the influence of epigenetics on multiple research areas has grown, the study of epigenetics has shifted from basic mechanisms to the effect of these mechanisms on development and disease. From developing the first magnetic ChIP kits to the first ChIP-chip kits, EMD Millipore has continued to develop and refine technologies for the study of epigenetic phenomena.

With a comprehensive portfolio, including the former Upstate® and Chemicon® product families, researchers can count on EMD Millipore's dependable, high quality reagents and expert support. Our dedicated research scientists develop assays and kits in-house, meaning that we can work with you and your specific research question to develop customized protocols that ensure successful studies of epigenetics and gene regulation.

Chromatin Assembly: Histones, Associated Proteins, and Antibodies

Chromatin is the complex of genomic DNA and associated proteins in the nucleus. Modifications to chromatin structure and the interplay of chromatin proteins play a direct role in epigenetic regulation. The structure of chromatin is facilitated by histones, a major class of chromatin proteins. Histones form the nucleosome, a complex containing 2 subunits each of histones H2A, H2B, H3 and H4. On the outside of the core complex, linker histone H1 occupies the internucleosomal DNA. This nucleosome complex maintains the compacted structure of chromatin. Site-specific histone modifications, such as methylation, acetylation, phosphorylation, ubiquitination, and citrullination, can alter local chromatin structure and regulate transcription, repair, recombination, and replication. Non-histone proteins associated with chromatin are a diverse group with thousands of different protein types, including transcription factors, polymerases, hormone receptors and other nuclear enzymes.

Description	Catalogue No.
Purified Proteins	
NAP1	14-837
Acf1/ISWI	14-836
Core Histones	13-107
Histone H1	14-155
Histone H2A, human	14-493
Histone H2B, human	14-491
Histone H2A.X	14-576
Histone H4	14-412
Antibodies	
Anti-ASF1a, clone MPH7	MABE90
Anti-BAF (BANF1)	09-893
Anti-CAF1 p150, clone SS1, 1-3	04-1522
Anti-CHD9	09-090
Anti-EZH 1/2, clone EP1408Y, Rabbit Monoclonal	04-1047
Anti-HP1γ, clone 42s2	05-690
Anti-hSNF2H	07-624
Anti-Mi-2	06-878
Anti-Mi-2b (CHD4)	06-1306
Anti-SNF2β/BRG1	07-478

EMD Millipore offers a range of kits, assays, recombinant proteins, and antibodies to enable the study of chromatin and histones.

Chromatin Proteins, Assembly, and Remodeling

All DNA-related processes function in chromatin rather than on naked DNA. Because transcriptional or post-transcriptional regulation on naked DNA can differ from that on assembled chromatin, it is crucial to have a reliable method for assembling and analyzing chromatin *in vitro*.

Chromatin Assembly Proteins

EMD Millipore's proteins for *in vitro* chromatin assembly include histone chaperone NAP1, which regulates chromatin fluidity, and Acf1/ISWI, a chromatin remodeling complex required for replication through heterochromatin. EMD Millipore's purified histones complete the assembly reaction. After combining NAP-1 with histones, followed by Acf1/ISWI, histones are deposited into nucleosome arrays in an ATP-dependent manner. The resulting DNA is perfect for *in vitro* transcription, chromatin immunoprecipitation, or analysis of the chromatin assembly process.

Chromatin Immunoprecipitation

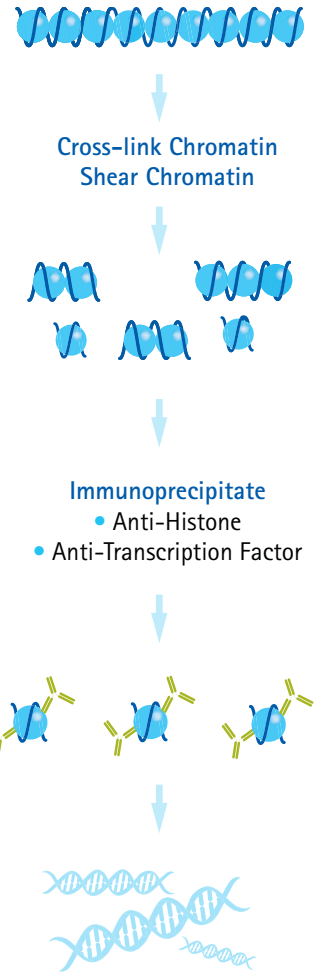
Chromatin immunoprecipitation (ChIP) is a powerful technique classically used for mapping the *in vivo* distribution of proteins associated with chromosomal DNA. These proteins can be histone subunits, transcription factors, or other regulatory or structural proteins bound either directly or indirectly to DNA. Successful ChIP requires high quality ChIP-validated antibodies that can specifically detect proteins associated with target regions of chromosomal DNA. Traditionally, endpoint and/or quantitative PCR (qPCR) are performed after ChIP to verify whether a particular DNA sequence is associated with the protein of interest. Using this classical approach, researchers can evaluate the interactions of the proteins of interest with a limited number of known target genes.

A History of Innovation

Upstate®, now part of EMD Millipore, launched the first ChIP kits in the 1990s. Since then, EMD Millipore has introduced an extensive line of ChIP technologies with many advantages:

- Improved sample prep
- One-day protocol
- High throughput ChIP
- Genome-wide analysis
- ChIP for tissues
- Optimized, specialized protocols
- Automation compatibility
- ChIP-validated antibodies
- Protein A, G, and A/G magnetic beads
- Alternate detection methods

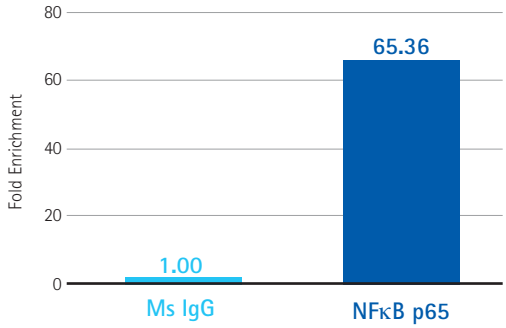
Chromatin IP Technique



One-Day ChIP Kits

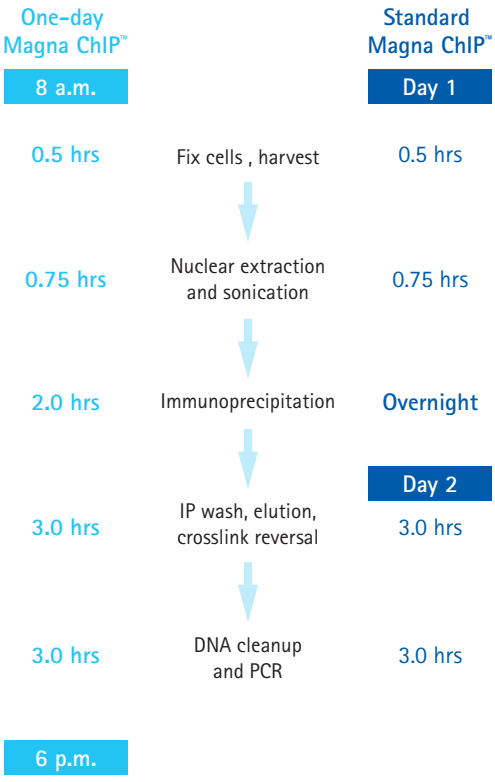
Magna ChIP™ and EZ-Magna ChIP™ Protein A/G Kits

- Complete ChIP in one day, from cells to PCR results*
- Protein A/G magnetic bead blend; enrichment of wider range of antibodies
- Suitable for a range of downstream applications
- Compatible with native ChIP
- EZ-Magna ChIP™ kit with essential positive and negative control antibodies, qPCR primers



Specific localization of NFκB binding via one-day ChIP using the EZ-Magna ChIP™ kit. Sonicated chromatin prepared from serum-starved, TNFα-treated HEK293 cells (~3 x 10^6 cell equivalents per IP) were subjected to chromatin immunoprecipitation using 4 µg of either Normal Mouse IgG, or 4 µg Anti-NFκB p65 (RelA) (components contained in NFκB p65 ChIPAb+™ kit (Catalogue No. 17-10060).

Immunoprecipitation of NFκB p65 (RelA)-associated DNA fragments was verified by qPCR using primers directed against IκBα.



Comparison of the One-day (Rapid) Magna Chip™ and Standard Magna ChIP™ protocols. The protocols vary primarily in the time required for immunoprecipitation. The Rapid Magna ChIP™ protocol is recommended primarily when using ChIP-validated antibodies against abundant targets. Use the Standard Magna ChIP™ protocol when using uncharacterized antibodies or for less abundant targets. Download the Magna ChIP™ user guide, 17-10086, for detailed protocols.

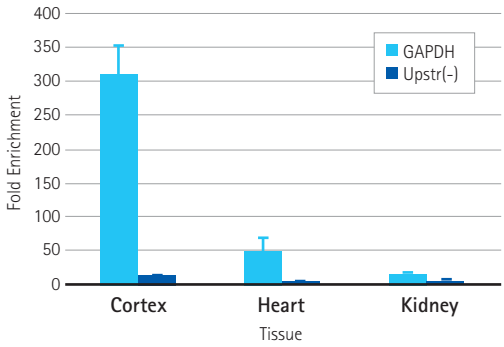
Description	Catalogue No.
Magnetic Bead-based Kits	
Magna ChIP™ A/G Kit	17-10085
EZ-Magna ChIP™ A/G Kit	17-10086
Magna ChIP™ A	17-610
EZ-Magna ChIP™ A	17-408
Magna ChIP™ G	17-611
EZ-Magna ChIP™ G	17-409
Agarose Bead-based Kits	
ChIP Assay Kit	17-295
EZ-ChIP™ Kit	17-371
Acetyl-Histone H3 Immunoprecipitation (ChIP) Assay Kit	17-245
Acetyl-Histone H4 Immunoprecipitation (ChIP) Assay Kit	17-229
ChIPAb+™ Validated Antibodies	(See Page 14)

Tissue ChIP

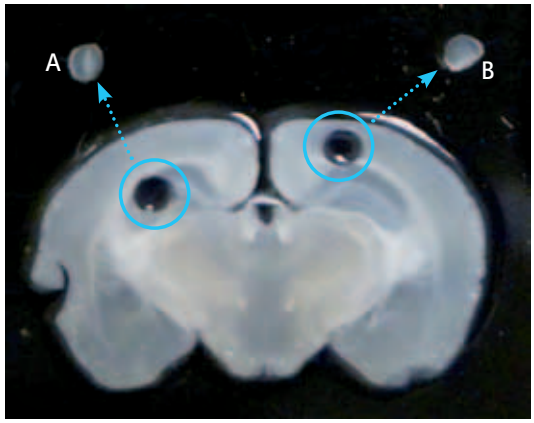
Magna ChIP™ G Tissue Kit

The Magna ChIP™ G Tissue Kit provides the tools necessary to obtain repeatable, reliable, and site-specific tissue biopsies.

- Reliable ChIP from a variety of tissue samples
- Microdissection punch for accurate tissue biopsy
- Complete set of optimized buffers
- Detailed, optimized protocol with guided workflow



Tissue-specific localization of RNA polymerase II to the GAPDH promoter as revealed using the Magna ChIP™ G Tissue Kit and Anti-RNA Polymerase II clone CTD4H8 (Catalogue No. 05-623B). 1 µg of antibody was used to immunoprecipitate chromatin from various mouse tissues. The resulting immunoprecipitated DNA was analyzed by qPCR with primers specific for the mouse GAPDH promoter. QPCR was used to amplify immunoprecipitated chromatin fragments and data were presented as fold relative enrichment to IgG-associated DNA from independent experiments. For a biological negative control, fold enrichment was assessed by qPCR with primers upstream of the Dhfr gene (UpStr (-)).



Region-Specific Tissue Isolation. A 300 µm coronal mouse brain cryosection was obtained and two microdissections were carried out using the 1 mm microdissection punch provided in the kit. The isolated tissue is shown placed above the dissected region: (A) hippocampus, (B) cortex.

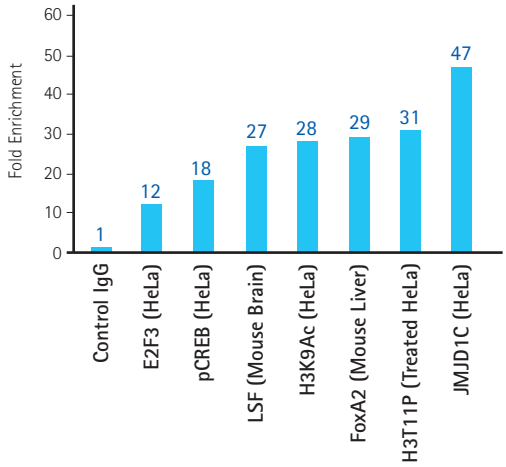
Description	Catalogue No.
Magna ChIP™ G Tissue Kit	17-20000
ChIPAb+™ Validated Antibodies	(See Page 14)

High Throughput (96-well) ChIP

Magna ChIP™ HT96 and EZ-Magna ChIP™ HT96 Kits

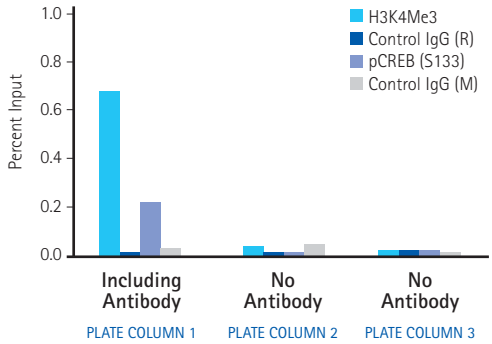
- Up to 96 ChIP reactions at once
- ChIP using cells or tissue
- Multichannel pipette or automated protocols
- Protein A/G magnetic bead blend
- EZ-Magna ChIP™ kit with essential positive and negative control antibodies, qPCR primers
- Efficient and reproducible
- Technically demanding ChIP made easy

Antibody Performance using Magna ChIP™ HT96 Panel 1



Chromatin was derived from sources indicated and subjected to immunoprecipitation with either specific ChIPAb+™ antibodies (x-axis) or with IgG, using the Magna ChIP™ HT96 multichannel pipette protocol. Assays were performed using conditions described in the respective ChIPAb+™ product user guides.

Minimal Well-to-well Carryover Contamination



Minimal well-to-well carryover contamination using automated protocol. Sonicated chromatin prepared from 100,000 untreated HeLa cells was subjected to chromatin immunoprecipitation using 1 µg of purified IgG (mouse IgG, Catalogue No.12-371B; Rabbit IgG, Catalogue No. 12-370) or specific antibodies (anti-H3K4Me3, Catalogue No.17-614; anti-Phospho-CREB, Catalogue No. 17-10131) and the Magna ChIP™ HT96 Kit using a Freedom EVO® robotic workstation. Immunoprecipitation of antibody-associated DNA fragments was verified by qPCR using control primers flanking the human GAPDH promoter region. Standard ChIP were performed in the first column of a 96-well plate, Mock IP without antibody were performed in the second and third column.

Description	Catalogue No.
Magna ChIP™ HT96	17-10077
EZ-Magna ChIP™ HT96	17-10078
Magna GrIP™ HT96 rack	17-10071
ChIPAb+™ Validated Antibodies	(See Page 14)

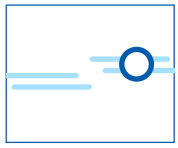
Genome-wide ChIP - Next Generation Sequencing

Magna ChIP-Seq™ Kit

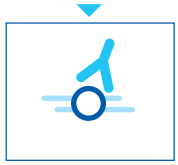
- Reliable ChIP-Seq library construction from as little as 1 ng of purified ChIP DNA
- Protein A+G bead blend is compatible with a broader range of antibodies
- Flexible format allows construction of single end, paired end, or barcoded libraries
- Sufficient reagents for up to 10 next generation sequencing library constructions
- Quality-controlled, validated enzymes and buffers in convenient master mix streamline library construction
- Includes validated positive and negative control antibodies and a control primer set
- Proven performance through construction and sequencing of genomic DNA libraries on an Illumina Genome Analyzer II
- Expert support from our highly trained technical support scientists

Description	Catalogue No.
Magna ChIP-Seq™ Chromatin Immunoprecipitation and Next Generation Sequencing Library Preparation Kit	17-1010

Magna ChIP-Seq™ Workflow

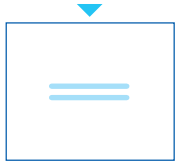


Isolate & Fragment Chromatin

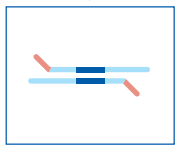


ChIP

Reverse Crosslinks

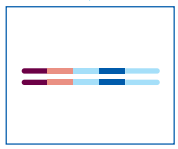


End Repair & Modification



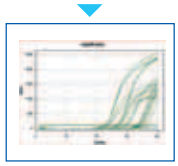
Adapter Ligation

Gel Isolation to Size - select Fragments



PCR Amplification

QC Library Using Microfluidic or Conventional Electrophoresis



QC Library by Real-time PCR

Next Gen Sequencing

Genome-wide ChIP - Microarray

Magna ChIP²™ DNA Microarray Kits

Magna ChIP²™ kits are an easy way to take your ChIP analysis genome-wide. These kits are the first and only complete solution that standardizes and simplifies ChIP-chip analysis by combining all necessary and fully optimized reagents with validated protocols and guidelines. Each Magna ChIP²™ kit is designed to ensure success, sensitivity, and reproducibility, using either Agilent® or user-provided DNA microarrays.

Magna ChIP²™ Universal Kits

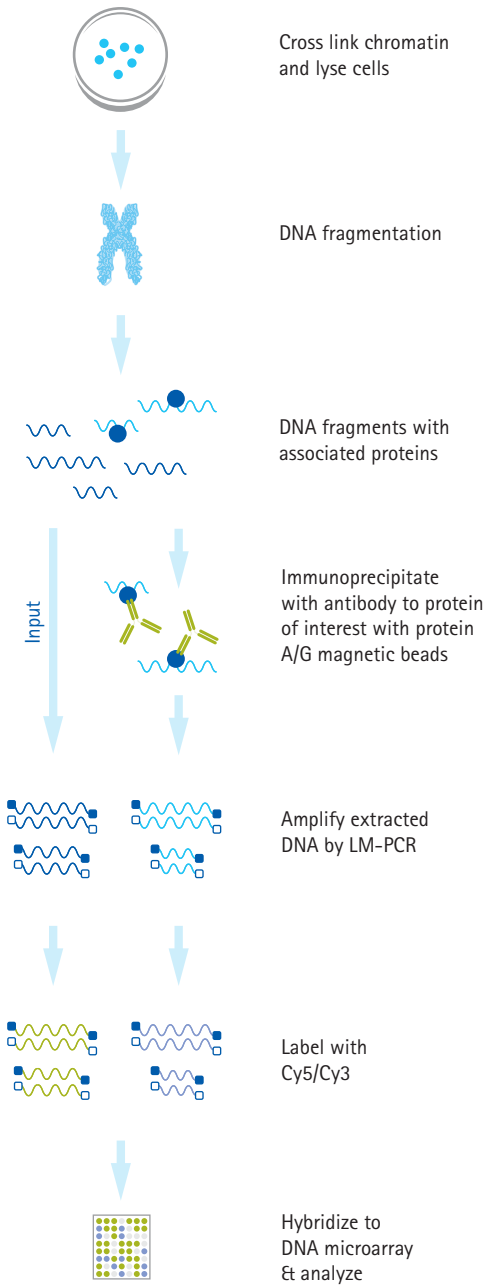
Perform ChIP-chip analysis on virtually any type of microarray with the universal Magna ChIP²™ kits. These kits contain optimized reagents and validated protocols for preparing chromatin that's ready for labeling and hybridization.

Magna ChIP²™ Human and Mouse Promoter Kits

Simplified ChIP-chip analysis with proven reagents and protocols for isolation, amplification, labeling and hybridization, including your choice of either human or mouse Agilent® promoter microarrays.

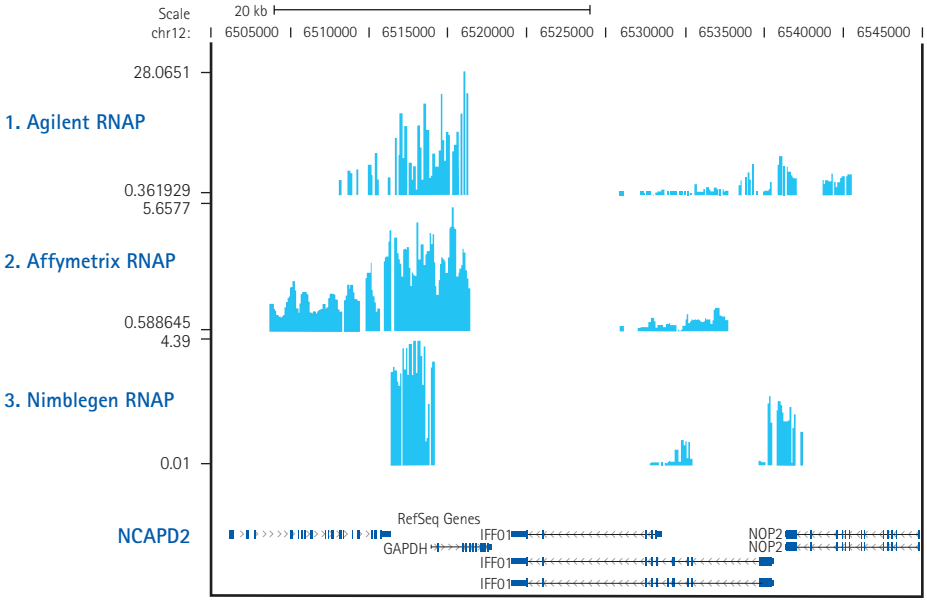
Description	Catalogue No.
Magna ChIP²™ Universal Kit (includes materials sufficient for 6 slides)	17-1000
Magna ChIP²™ Universal Quad Kit (includes materials sufficient for 24 slides)	17-1004
Magna ChIP²™ Human Promoter Kit (includes materials sufficient for 6 slides)	17-1001
Magna ChIP²™ Mouse Promoter Kit (includes materials sufficient for 6 slides)	17-1002

ChIP-chip Workflow



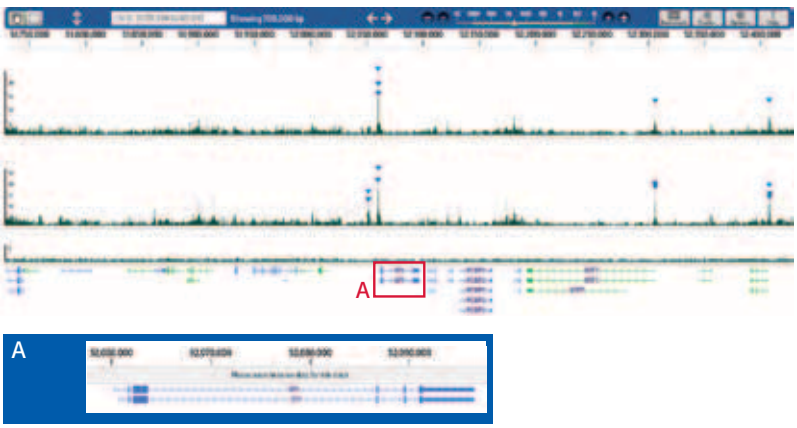
Genome-wide ChIP - Sample Data

Comparison of commercially available arrays using Magna ChIP²™ Universal Kit



Magna ChIP²™ kits enable genome-wide ChIP analysis using multiple types of microarrays. Comparative results for the Agilent human 244K promoter array (Top), Affymetrix® human promoter array (Middle) and Nimblegen™ human promoter array (Bottom) using the EMD Millipore Magna ChIP²™ kit.

Next gen sequencing analysis of Sp1-associated DNA library prepared using the Magna ChIP-Seq™ Kit



Effective ChIP and Reliable Next Gen Sequencing Library Construction from Limited Amounts of DNA. Sequencing libraries were constructed using the Magna ChIP-Seq™ Kit (Catalogue No. 17-1010) and the ChIPAb+™ Sp1 antibody/primer set (Catalogue No. 17-601). Libraries were constructed using 1ng, 10ng or an input chromatin sample and sequenced using an Illumina Genome Analyzer. Peak analysis (derived using quantitative enrichment of sequence tags (QuEST)) of the Sp1 locus from confidently mapped reads browsed with DNAnexus™ software shows Sp1 binding (triangles) occurs near expected Sp1 binding sites.

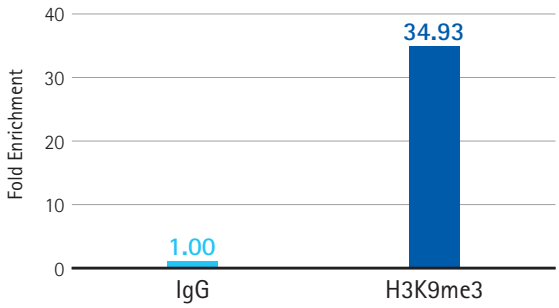
ChIP-Validated Antibodies

ChIPAb+™ Antibody and Primer Sets

Antibody recognition in the context of chromatin can differ from other immunoassays. Avoid ChIP failure due to poor antibody performance by using ChIPAb+™ antibodies. To ensure reliable performance in your lab, each lot is individually validated and tested for ChIP.

ChIPAb+™ kits are more than just an antibody. Each set also includes a negative control antibody, plus control primers for amplifying a known, enriched locus to help you validate your results.

ChIPAb+™ trimethyl-histone H3 (Lys9)



ChIPAb+™+ trimethyl-histone H3 (Lys9) (17-625): Sonicated chromatin from NIH 3T3 L1 cells was subjected to chromatin immunoprecipitation using either normal rabbit IgG or Anti-trimethyl-histone H3 (Lys9) antibody and the Magna ChIP™ A Kit (17-610). Successful enrichment of trimethyl-histone H3 (Lys9)-associated DNA fragments was verified by qPCR using primers flanking the mouse p16 promoter.

Description	Catalogue No.
ChIPAb+™ Histone H2A.Z	17-10048
ChIPAb+™ Histone H2B	17-10054
ChIPAb+™ Histone H3 (C-term)	17-10046
ChIPAb+™ Histone H3 (Unmod Lys4)	17-675
ChIPAb+™ Acetyl Histone H3	17-615
ChIPAb+™ Acetyl-Histone H3 (Lys4)	17-10050
ChIPAb+™ Acetyl-Histone H3 (Lys9)	17-658
ChIPAb+™ Acetyl-Histone H3 (Lys14)	17-10051
ChIPAb+™ Monomethyl Histone H3 (Lys27)	17-643
ChIPAb+™ Dimethyl-Histone H3 (Lys4)	17-677
ChIPAb+™ Dimethyl-Histone H3 (Lys9)	17-648
ChIPAb+™ Trimethyl-Histone H3 (Lys4)	17-614
ChIPAb+™ Trimethyl-Histone H3 (Lys4)	17-678
ChIPAb+™ Trimethyl-Histone H3 (Lys9)	17-625
ChIPAb+™ Trimethyl-Histone H3 (Lys27)	17-622
ChIPAb+™ Trimethyl-Histone H3 (Lys36)	17-10032
ChIPAb+™ Trimethyl-Histone H3 (Lys79)	17-10130
ChIPAb+™ Phospho-Histone H3 (Ser10)	17-685
ChIPAb+™ Acetyl Histone H4	17-630
ChIPAb+™ Acetyl-Histone H4 (Lys5)	17-10045
ChIPAb+™ CREB	17-600
ChIPAb+™ CTCF	17-10044
ChIPAb+™ EED	17-663
ChIPAb+™ EED (Rabbit Polyclonal)	17-10034
ChIPAb+™ ERα	17-603
ChIPAb+™ EZH2, clone AC22	17-662
ChIPAb+™ HDAC1	17-608
ChIPAb+™ p53	17-613
ChIPAb+™ Phospho-CREB (Ser133)	17-10131
ChIPAb+™ REST	17-641
ChIPAb+™ RNA Polymerase II	17-620
ChIPAb+™ SMRT	17-10057
ChIPAb+™ Sox-2, clone 6F1.2	17-656
ChIPAb+™ Sp1	17-601
ChIPAb+™ SUZ12	17-661
ChIPAb+™ TATA Binding Protein (TBP)	17-10098

For a complete list of our epigenetics products, please visit:
www.millipore.com/epigenetics

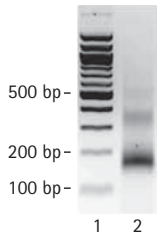
Magnetic Beads

Magna ChIP™ magnetic beads with protein A, G, or A/G are optimized specifically for ChIP applications and are a rapid, reproducible, and efficient reagent for collecting immunocomplexes in ChIP assays. Unlike conventional agarose beads, Magna ChIP™ magnetic beads are rapidly moved to the side of a reaction vessel when exposed to a magnetic field, and significantly reduce the handling time and mechanical stress on target immunocomplexes.

EZ-Zyme™ Chromatin Preparation Kit

- No sonication
- Mild and efficient fragmentation of chromatin
- Compatible with native ChIP

Chromatin from formaldehyde-crosslinked HeLa cells was prepared and digested with EZ-Zyme™. Digested chromatin (lane 2) was electrophoresed through a 2% agarose gel and stained with ethidium bromide. Lane 2 shows that the majority of the chromatin has been digested to lengths of mono- and dinucleosomes. DNA size markers are in lane 1.



Description	Catalogue No.
EZ-Zyme™ Chromatin Preparation kit	17-375
Magna ChIP™ Protein A+G Magnetic Beads	16-663
Magna ChIP™ Protein G Magnetic Beads	16-662
Magna ChIP™ Protein A Magnetic Beads	16-661
Magna GrIP™ Rack (8-well)	20-400
PureProteome™ Magnetic Stand (8 x 1.5 or 2 mL, removable magnet)	LSKMAGS08
Magna GrIP™ HT96 Rack	17-10071

Magnetic Racks for ChIP Assays

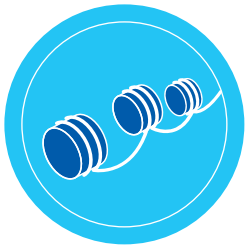
Choose one of our magnetic racks for Magna ChIP™ assays: the classic Magna GrIP™ rack, the extra-strong, contoured PureProteome™ magnetic stands, or the new Magna GrIP™ HT96 rack, which is ideal for high throughput ChIP.

PureProteome™ Magnetic Stand

- Effective bead capture: Strong trapezoid-shaped magnet fits tube contours to capture up to 300 µL of beads
- Efficient agitation: Removable magnet and unique vortex interface enables thorough mixing
- Easy to handle: Ergonomically designed magnetic stand securely holds both 1.5 mL and 2 mL tubes

Magna GrIP™ Rack

- Effective bead capture: polyethylene rack containing 4 neodymium magnets
- Versatile: rack is versatile, and may also be used with either 15 mL or 0.5 mL tubes.
- Easy to handle: Ergonomically designed magnetic stand has 8 holes suitable for 1.5-2.0 mL tubes or spin columns



Histone Modifications

The most commonly studied and best understood histone modifications are acetylation, phosphorylation, methylation, and ubiquitination. Histone modifications regulate DNA transcription, repair, recombination, and replication, and can alter local chromatin architecture.

EMD Millipore offers a wide range of antibodies rigorously tested for specificity in dot blot analyses, as well as recombinant proteins, and kits for analyzing complex histone modification patterns.

Acetylation

Histone acetylases (HATs) and deacetylases (HDACs) are key regulators of gene expression and function. Transcription activation complexes contain HATs, which acetylate histone lysines and open chromatin structure to permit transcription. HDACs remove acetyl groups, leading to decreased gene expression.

Methylation

Methylation of certain histone residues is strongly indicative of euchromatin and transcriptional activation, while other methylation events are hallmarks of heterochromatin and correlate with transcriptional repression. Histone methylation can be reversed by site-specific histone demethylases, such as LSD1, UTX, and the JMJD family of enzymes. The coordinated activity of histone methylases and demethylases temporally and spatially regulates gene expression, particularly during embryonic development.

Phosphorylation

Phosphorylation of histones commonly occurs during chromosome condensation in mitosis, in cells undergoing apoptosis and in response to DNA damage. However, certain histone sites are phosphorylated in response to very early gene induction signaling indicating that, depending on site and cellular context, histone phosphorylation may promote either opening or closing of chromatin structure.

Ubiquitination

Ubiquitination is required for certain histone methylation events and involves ubiquitin-activating enzymes (E1s), ubiquitin-conjugating enzymes (E2s), and ubiquitin-protein ligases (E3s). Our wide range of products for measuring ubiquitination includes unique antibodies for specific ubiquitin linkages and modified histone residues.

Citrullination

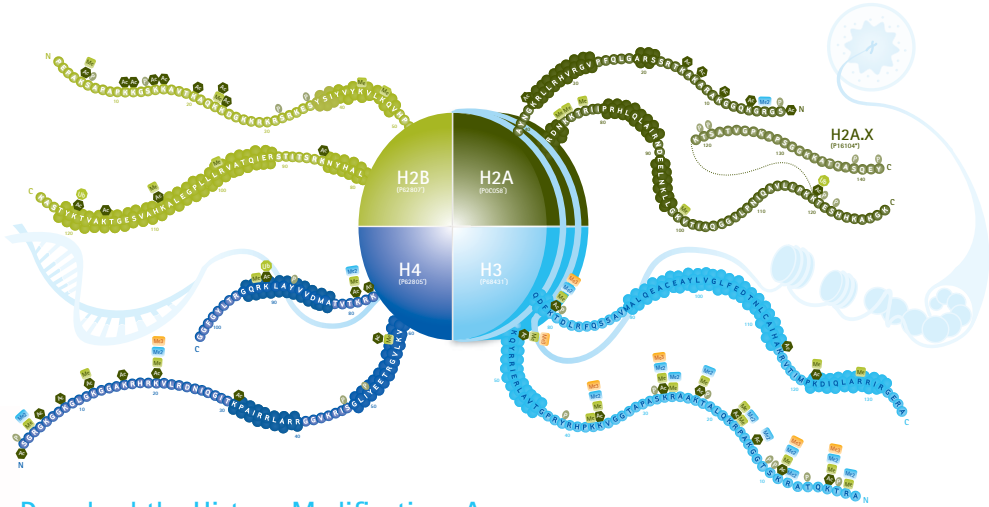
Citrullination is a modification of arginine that may play a role in rheumatoid arthritis and multiple sclerosis. 10% of histones are citrullinated, suggesting that citrulline has a role in gene regulation. EMD Millipore offers a site-specific antibody to citrullinated histone H4, as well as antibodies and assays to detect this unique modifications.

Availability of Antibodies to Histone Modifications

For a complete product listing visit: **www.millipore.com/antibodies**

	H1	H2A	H2B	H3	H4
Unmodified	Yes	Yes	Yes	Yes	Yes
Acetyl		Any, K5, K7, K9	K5, K12, K15, K16, K20, K120	Any, K4, K9, K14, K18, K23, K27, K36, K56, K79	Any, K5, K8, K12, K16
Monomethyl				K4, K9, K27, K36	K20
Dimethyl			K5, K11	K4, K9, K14, K23, K27, K36, K37, K79, R2, R17, R26	K20, K79, R3
Trimethyl				K4, K9, K27, K36, K79, K23	K20
Any methyl				K4,	
R17	K20				
Phospho	Any	S1, S129	S14	S10, S28, S31, T3,	
T11, T22, T80,	Any				
Ubiquityl		K118	K120		
Citrulline					R3

Table 1. Some of the specific histone modifications detected by EMD Millipore antibodies. For each histone protein (indicated in the top row), the different types of modifications are listed (leftmost column) and specific modified amino acids to which EMD Millipore antibodies are available are indicated in the table using the single letter amino acid abbreviations and number to represent the position in the sequence. Where applicable, antibodies also recognize modifications on any amino acid on a histone protein (Any) or unmodified protein.

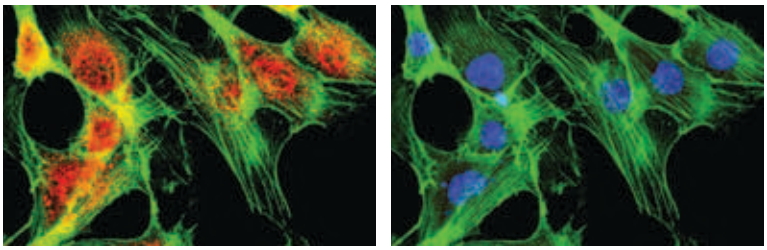


Download the Histone Modifications App

for ready access to the biological significance and epigenetic implications of core histone amino acid modifications.

Available on the App Store

Description	Catalogue No.
Acetylation	
HDAC1 Active recombinant protein	14-838
HDAC4 Active recombinant protein	14-828
HDAC7 Active recombinant protein	14-832
Histone Acetyltransferase Inhibitor IV, CPTH2	382111
Histone Acetyltransferase p300 Inhibitor, C646	382113
HDAC Inhibitor XXII, NCH51	382185
HDAC Inhibitor XXIV, OSU-HDAC-44	382181
Histone Deacetylase Inhibitor VII, 106	382173
SIRT1 Inhibitor III	566322
SIRT1 Inhibitor IV, (S)-35	566325
SIRT1/2 Inhibitor VIII, Salermide	566330
SIRT2 Inhibitor, AGK2	566324



Confocal IF analysis of NIH 3T3 cells using anti-ubiquitin (Lys48-specific) (red). Actin filaments were labeled with Alexa Fluor®-488-Phalloidin (green). Nuclear material is stained with DAPI (blue).

Description	Catalogue No.
Methylation	
Anti-Monomethyl-Histone H3 (Lys4)	07-436
Anti-Dimethyl-Histone H3 (Lys4)	07-030
Anti-Trimethyl-Histone H3 (Lys4)	05-745R
Anti-Monomethyl-Histone H3 (Lys27)	07-448
Anti-Dimethyl-Histone H3 (Lys27)	07-452
Anti-Trimethyl-Histone H3 (Lys27)	07-449
Anti-Monomethyl-Histone H4 (Lys20), clone NL314	04-735
Anti-Dimethyl-Histone H4 (Lys20)	07-1584
Anti-Trimethyl-Histone H4 (Lys20)	07-463
Anti-LSD1	09-058
Anti-JMJD3	07-1533
LSD1 Inhibitor	489476
LSD1 Inhibitor II, S2101	489477
Histone Lysine Methyltransferase Inhibitor	382190
Protein Arginine N-Methyltransferase Inhibitor, AMI-1	539209
Protein Methyltransferase Inhibitor, AMI-5	539211
JMJD2 Inhibitor, 5-carboxy-8HQ	420201
H CARM1, active	14-575
PRMT1, active	14-474
PR-SET7, active	14-539
SET9	14-469
Phosphorylation	
JAK2 Inhibitor II	420132
JAK2 Inhibitor III, SD-1029	573098
AMPK Inhibitor, Compound C	171260
Ubiquitination	
Anti-Ubiquityl-Histone H2A, clone E6C5	05-678
Anti-Ubiquitin, Lys48-Specific, clone Apu2	05-1307
Ubiquitin Activating Enzyme E1	14-857
UbcH2 Conjugating Enzyme	14-807
hHR6B Conjugating Enzyme	14-854
Ubiquitin E1 Inhibitor, PYR-41	662105
Hdm2 E3 Ligase Inhibitor	373225
Citrullination	
Anti-Citrulline	AB5612
Anti-Histone H4 (citrulline 3)	07-596

For a complete selection of histone antibodies, recombinant proteins, enzymes, and inhibitors, please visit: **www.millipore.com/epigenetics**

Histone Deacetylase (HDAC)

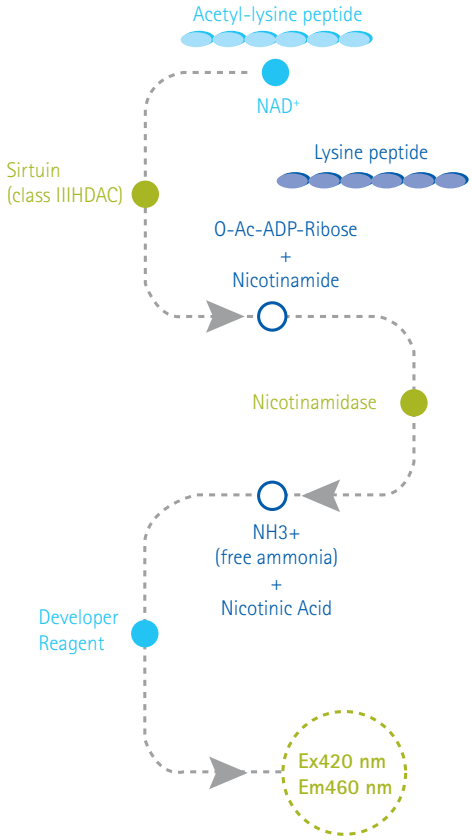
SIRTainty™ Class III HDAC Assay

Sirtuins (class III HDACs) became the focus of intense research when it was discovered that their activation led to reduced incidence of aging and age-related diseases, including diabetes.

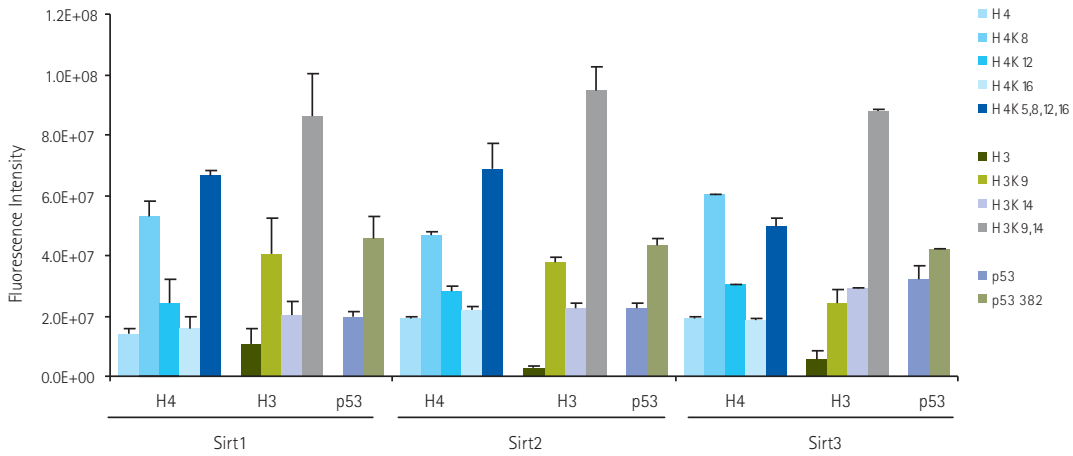
The SIRTainty™ Assay Features

- **Sensitive**: Almost five times more sensitive than assays dependent on labeled substrates.
- **Flexible**: Analyze multiple sirtuin isoforms using virtually any substrate.
- **Reliable**: Avoid fluorophore-mediated activation by compounds such as resveratrol.
- **Fast and Easy**: Homogeneous, no-wash, 96-well assay minimizes hands-on time.

Description	Catalogue No.
SIRTainty™ Class III HDAC Assay	17-10090
SIRT1 Deacetylase	17-370
HDAC Assay Kit, Colorimetric	17-374
HDAC Assay Kit, Fluorometric	17-356



Sirtuin-mediated deacetylation of unlabeled peptide substrate generates nicotinamide as a product. The SIRTainty™ assay couples sirtuin enzyme activity to nicotinamidase, which cleaves nicotinamide into nicotinic acid and free ammonia. A developer reagent is added, which reacts with the free ammonia to generate a fluorophore. The resulting fluorescent signal is quantified with a conventional fluorometric plate reader.

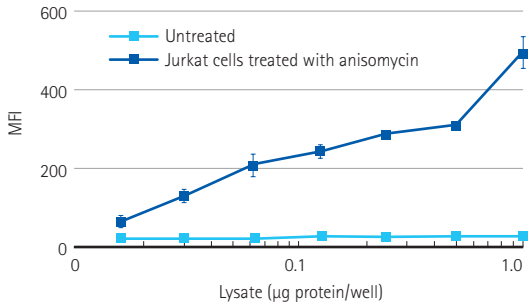


Sirt1, Sirt2, and Sirt3 exhibit preference for acetylated vs. nonacetylated peptides.

H2A.X Phosphorylation

MILLIPLEX® MAP Human Phospho-Histone H2A.X (Ser139) MAPmate™ Kit

Phosphorylated H2A.X (Ser139) is the key component of the signal transduction pathways that are mobilized during DNA damage.The MILLIPLEX® MAP phospho-histone H2A.X (Ser139) MAPmates™ contain xMAP® beads conjugated to anti-H2A.X and biotinylated anti-phospho-H2A.X, designed for bead-based multiplex measurement of phosphorylated histone H2A.X (Ser139) in cell lysates using Luminex® instruments.

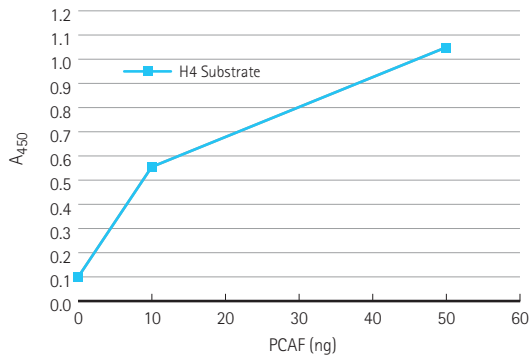


MILLIPLEX® MAP detection of changes in phosphorylation of histone H2A.X (Ser139) in Jurkat cells stimulated with or without 25 mM anisomycin. The Median Fluorescent Intensity (MFI) was measured using the Luminex® instrument.

Histone Acetyltransferase ELISA

HAT Assay Kit

- 96 assays in each kit
- Biotinylated histone peptides
- Detects acetylated lysine on histones H3 or H4
- Acetylated H3 and H4 peptides included for controls
- Fully optimized buffers



HAT Assay (17-289): Biotinylated histone H4 peptide was acetylated for 30 minutes with 10-50 ng of recombinant PCAF (14-309) in the presence of 100 mM acetyl-CoA and 1X HAT assay buffer.

Description	Catalogue No.
MILLIPLEX® MAP Human Phospho-Histone H2A.X (Ser139) MAPmate™ Kit	46-692
H2A.X Phosphorylation Assay Kit (Flow cytometry)	17-344
Anti-Citrulline (Modified) Detection Kit	17-347
HAT Assay Kit	17-289
Histone Methyltransferase Assay Reagent Kit	17-330

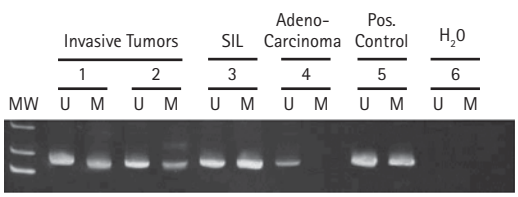
DNA Methylation

DNA methylation is involved in the regulation of many cellular processes, including chromosome stability, chromatin structure, X chromosome inactivation, embryonic development, and transcription.

The discovery that differences between genomes cannot fully explain phenotypic differences between species or even between individuals has spurred the sequencing of "methylome" data sets consisting of the location of every methylated cytosine in an organism's genomic DNA. Advances in methylated DNA mapping, together with increased access to high resolution DNA sequencing, has made possible the large number of recently published "methylomes" in species ranging from rice to sea squirts, and in the presence of diverse environmental signals.

EMD Millipore offers a wide selection of CpG WIZ® gene-specific PCR kits for isolation of methylated DNA, and a growing portfolio of antibodies to important enzymes and markers including DNA methyltransferases, TET, and 5-hydroxycytosine.

Methylation-specific PCR (MSP) is an established technique for mapping and monitoring methylation patterns in the CpG islands of genomic DNA. EMD Millipore's CpGenome™ and CpG WIZ® systems allow sensitive detection of gene methylation using MSP of bisulfite-modified DNA.



Detection of the Methylation State of the p16 Gene. MSP of the p16 gene in two invasive carcinomas, a squamous intraepithelial lesion (SIL), and an adenocarcinoma of the cervix. Both invasive carcinomas and the SIL sample are heterozygous for methylation while the adenocarcinoma sample is homozygous for the unmethylated state at the p16 locus.

CpG Islands

About 1% of the genome consists of 500-2000 bp CpG-rich areas or islands. About half of all CpG islands correspond to transcription start sites and promoters of expressed genes. Methylation of CpG islands occurs on cytosine residues at position 5, to form 5-methylcytosine (5mC), which is thought to be an important mechanism for gene silencing in embryonic development, and inactivation of defined tumor suppressor genes in human cancers.

5-hydroxymethylcytosine and TET

5-hydroxymethylcytosine (5hmC) is generated from 5mC by the family of Ten-Eleven Translocation (TET1-3) enzymes and may also play a critical role in epigenetic gene regulation. 5hmC residues are found in active genes and are emerging as regulators of gene activation and cellular differentiation during embryonic development and brain maturation. Relatively high levels of 5hmC have been detected in the brain, especially in certain areas, such as the hippocampus, that are required for cognitive functioning. 5hmC and TET enzymes may also be involved in tumorigenesis, and are therefore key targets for epigenetics research, to fully elucidate the dynamic changes in the epigenome involved in development and disease.

DNA Methyltransferases

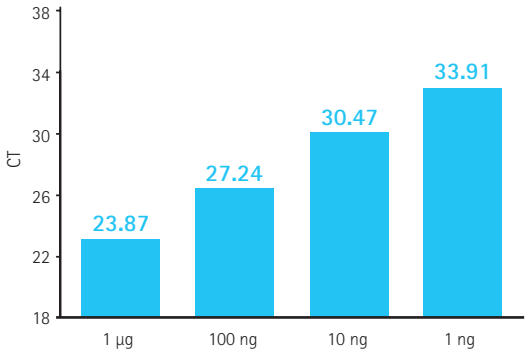
Key to epigenetic regulation is the family of DNA methyltransferase enzymes: DNMT1, DNMT3a, and DNMT3b. These enzymes maintain specific patterns of DNA methylation, and regulate the activity of a growing number of methyl-binding proteins, including MECP2, MBD1, MBD2, MBD3, MBD4, and Kaiso, which bind to methylated DNA and may function as methylation-sensitive transcriptional repressors or activators.

Bisulfite Modification

CpGenome™ Turbo Bisulfite Modification Kit

- Simple, 90-minute protocol
- Conversion efficiencies of 99.9% without over-conversion
- Accommodates a range of input sample amounts from 1 ng to 1 µg
- Recover modified DNA in as little as 25 µL final volume
- Proven performance in multiple downstream applications
- Optimized protocol enables virtually complete conversion of input samples while minimizing DNA damage
- Spin-column–based desulfonation and isolation procedure promotes efficient recovery of modified DNA ready for downstream analysis

Reliable Performance Across a Range of Input Sample Amounts



Sensitive and Reliable Bisulfite Conversion from 1 ng to 1 µg Methylated DNA (Catalogue No. S7821) was bisulfite treated as described in the CpGenome™ Turbo protocol and eluted in 50 µL (1 µg and 100 ng samples) or in 25 µL (10 ng and 1 ng samples). Conversion was evaluated by quantitative PCR using the CpG WIZ® MGMT methylated primer set (Cat. No. S7803).

Rapid, Efficient, and Specific Bisulfite Conversion Without Over-Conversion

```
GGTGCGCACCGTTTGCGACTTGGTGAGTGTCTGGGT
----C-T-TC-----C--T----------T-----
----C-T-TC-----C--T----------T-----
----C-T-TC-----C--T----------T-----
----C-T-TC-----C--T----------T-----
----C-T-TC-----C--T----------T-----
```

Complete conversion of unmethylated cytosines without conversion of methylated cytosines DNA containing methylated CpG cytosines (blue) and non-CpG cytosines (green) was bisulfite converted using the CpGenome™ Turbo bisulfite modification kit. Using MGMT methylation-specific primers from the CpG Wiz® MGMT primer set (Catalogue No. S7803), amplicons for DNA sequencing were generated. Alignments of the resulting sequences are shown below. In all clones examined, unmethylated cytosines were completely converted while methylated cytosines were unconverted.

Description	Catalogue No.
CpGenome™ Turbo Bisulfite Modification Kit	S7847
CpGenome™ Universal DNA Modification Kit	S7820
CpG WIZ® BRCA1 Amplification Kit	S7830
CpG WIZ® DAP-Kinase Amplification Kit	S7801
CpG WIZ® E-Cadherin Amplification Kit	S7804
CpG WIZ® ERα Amplification Kit	S7815
CpG WIZ® Fragile X Amplification Kit	S7807
CpG WIZ® hMLH1 Amplification Kit	S7811
CpG WIZ® MGMT Amplification Kit	S7803
CpG WIZ® Oct-4	S7840
CpG WIZ® p16 Amplification Kit	S7800
CpG WIZ® Prader-Willi/Angelman Amplification Kit	S7806
CpG WIZ® RASSF1A Amplification Kit	S7813
CpG WIZ® RB1 Amplification Kit	S7810

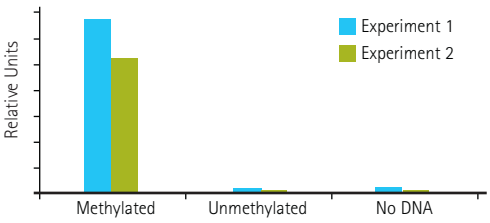
For a complete product listing visit:
www.millipore.com

Methylated DNA Enrichment

CpG MethylQuest™ DNA Isolation Kit

- Specific enrichment of methylated DNA fragments
- No detectable binding of unmethylated or hemimethylated regions
- Simple and fast 2-hour magnetic bead-based protocol
- GST-MBD2b capture protein pre-bound to magnetic beads for consistent results
- Reliable performance from 1 ng to 1 µg of DNA
- Elute ready-to-use DNA; avoid additional cleanup steps that reduce yields

Specific Binding of Methylated DNA

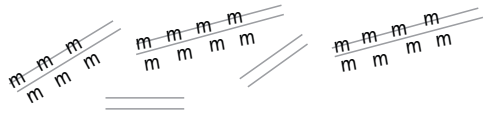


CpG MethylQuest™ MBD protein binds methylated DNA, but not unmethylated DNA. CpG MethylQuest™ protein was incubated with fully methylated or unmethylated p16 amplicons immobilized on magnetic beads, or a no-DNA control. Beads were washed and CpG MethylQuest™ protein was detected with an anti-GST antibody-horseradish peroxidase conjugate). The bars represent duplicate experiments.

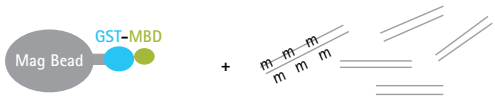
Description	Catalogue No.
CpG MethylQuest™ DNA Isolation Kit	17-10035
CpG MethylQuest™ Protein	14-921

Rapid and Simple CpG MethylQuest™ Protocol

1. Fragment genomic DNA by restriction digestion.



2. Add Fragmented DNA to CpG MethylQuest™ Beads.



3. Incubate to allow binding of methylated DNA.

4. Remove or collect supernatant containing unmethylated DNA.

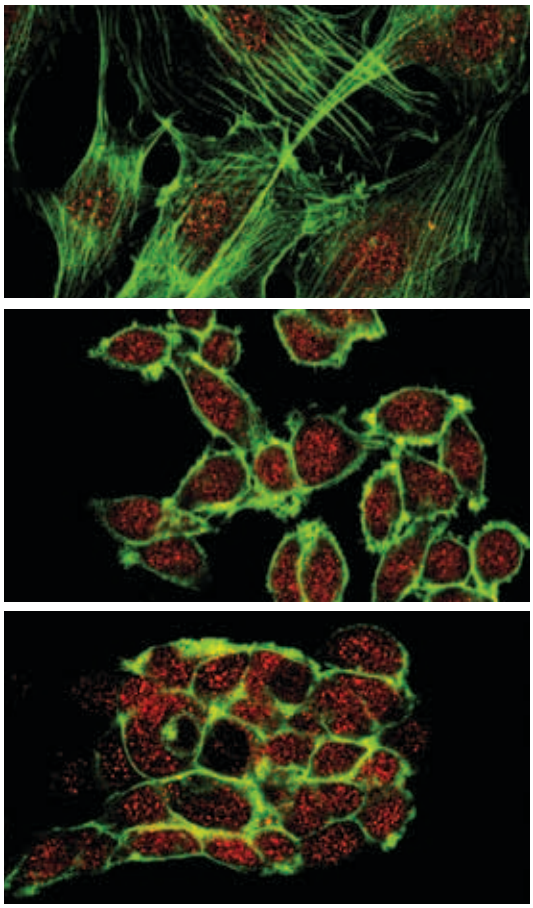
5. Wash beads and heat to elute methylated DNA.

DNA METHYLATION: ANTIBODIES AND INHIBITORS

EMD Millipore offers a growing selection of high performance antibodies and inhibitors for the study of DNA methyltransferases, methyl-binding proteins, 5-hydroxymethylcytosine, and TET.

Anti-DNMT3A2

DNMT3A2 is a developmentally regulated DNA methyltransferase and is known to induce *de novo* methylation in embryonic stem cells.



Nuclear localization of DNMT3A2 as shown by confocal IF analysis of NIH 3T3 (top), HeLa (middle), or A431 (bottom) cells using a 1:100 dilution of anti-DNMT3A2 polyclonal antibody (Red). Actin filaments have been labeled with Alexa Fluor® 488 -Phalloidin (Green).

Description	Catalogue No.
5-Methylcytosine, 5-Hydroxymethylcytosine, and TET	
Anti-5-Hydroxymethylcytosine, clone AB3/63.3	MABE176
Anti-Methylcytosine Dioxygenase TET1	09-872
Anti-5-Methylcytosine Mouse mAb (162 33 D3)	NA81-50UG
Anti-5-Methylcytosine, clone 33D3	MABE146
DNA Methyltransferases	
Anti-DNA Methyltransferase 3a (86-100) Rabbit pAb	317282-100UG
Anti-DNA Methyltransferase 1	AB3429
Anti-DNA Methyltransferase 3b	AB3433
Anti-DNA Methyltransferase 3a	AB3431
Anti-DNMT3A2	07-2050
Anti-DNMT1	07-688
Anti-DNMT-1 Mouse mAb (60B1220.1)	ST1133-50UG
DNA Methyltransferase Inhibitor	260920
DNA Methyltransferase Inhibitor II, SGI-1027	260921
Anti-CFP1	ABE211
Anti-DMAP1, C-terminus	07-2072
CpG-Binding Proteins	
Anti-Phospho-DNMT1(Ser714)	07-1594
Anti-MBD1 (methyl-CpG binding domain) protein 1	09-833
Anti-MBD4	07-2057
Anti-MBD1, C-terminus	07-2054
Anti-MBD2	07-198
Anti-acetyl-MeCP2 (Lys464)	ABE28
Anti-MeCP2 (Rabbit Polyclonal)	07-013
Anti-MeCP2 (Chicken Polyclonal)	ABE171
Related Antibodies	
Anti-Kaiso (659-672) Goat pAb	PC723-100UG
Anti-Kaiso, clone 6F	05-659
Anti-CBX-4, clone 10H10.2	MAB11012

For a complete selection of antibodies and proteins for DNA methylation, please visit: **www.millipore.com/epigenetics**

KOD DNA Polymerases

KOD DNA polymerases meet the demands of epigenomic PCR analysis. KOD Hot Start DNA polymerase effectively amplifies CpG island regions following MSP[1].

KOD Xtreme™ Hot Start DNA polymerase efficiently amplifies promoter regions with up to 90% GC-rich DNA. This enzyme can be used to amplify gene targets from crude tissue lysates. Unlike many polymerases, KOD Xtreme™ polymerase is not limited to low alkaline pH following bisulfite treatment of DNA during methylation-specific PCR (MSP).

Reference:

1. Hirai et al, Down-Regulation of Connexin 32 Gene Expression through DNA Methylation in Human Renal Cell Carcinoma. Am J Nephrol 2003; vol 23: 172-177.

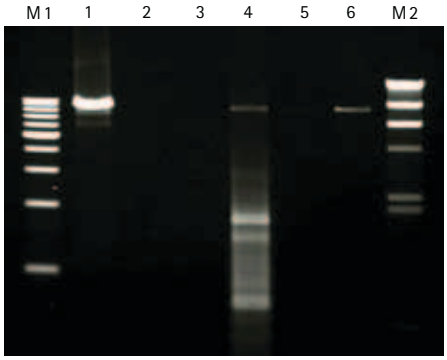
Thermocycling Conditions

94 °C for 2 minutes	1 cycle
98 °C for 1 s 74 °C for 1min/kb	5 cycles
98 °C for 1 s 72 °C for 1 min/kb	5 cycles
98 °C for 10 s 70 °C for 1min/kb	5 cycles
98 °C for 10 s 68 °C for 1 min/kb	15 cycles

Competitor polymerase PCR reactions were set up and thermocycling was performed according to manufacturers' protocols.

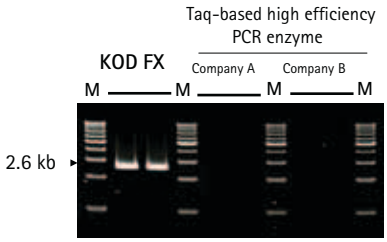
Description	Catalogue No.
KOD DNA Polymerase	71085-3
KOD Hot Start DNA Polymerase	71086-3
KOD Hot Start DNA Polymerase	71086-4
KOD Hot Start Master Mix	71842-3
KOD Hot Start Master Mix	71842-4
KOD XL DNA Polymerase	71087-3
KOD Xtreme™ Hot Start DNA Polymerase	71975-3

Amplify GC-Rich DNA with KOD Xtreme™ Hot Start DNA Polymerase



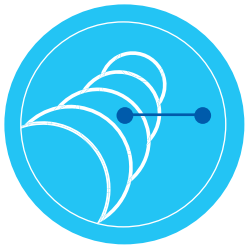
KOD Xtreme™ Hot Start DNA Polymerase amplifies GC-rich targets more efficiently than other polymerases. Six polymerases were used to amplify a 8.9 kb human IGF2R gene, containing ~90% GC content. Lane M1 and M2, markers; Lane 1, PCR using KOD Xtreme™ Hot Start DNA Polymerase (thermocycling as shown); Lanes 2 to 6, competitor polymerase systems supplied with GC Buffers and tested using manufacturer protocols. Data contributed by Akio Sugiyama, Tsuruga Institute of Biotechnology.

KOD Xtreme™ Gene Target Amplification from Challenging Tissues Using Minimal Alkaline Lysis



M: 1 kb Ladder Marker

KOD Xtreme™ polymerase efficienty amplifies DNA from tissues subjected to alkaline lysis vs. other polymerases. Three polymerases were used to amplify a 2.6 kb membrane glycoprotein (Thy-1) gene from mouse tail lysates. Alkaline lysates of mouse tail were neutralized, centrifuged, and 1 µL used for amplification using KOD Xtreme™ Hot Start DNA Polymerase. The reaction was incubated at 94°C for 2 min (polymerase activation) followed by thermocycling for 30 cycles at 98°C for 10s (denaturation), annealing and extension at 68°C for 1 min. Competitor polymerase PCR reactions were performed according to manufacturers' protocols.



Transcriptional and Post-Transcriptional Control

Traditionally, gene expression research has focused on transcriptional regulation through the interactions of transcription factors with specific binding sites, modifications of histones within chromatin, and coordinate chromatin dynamics associated with changes in gene transcription. Although those processes are still a central part of epigenetics research, more focus has been directed to RNA in recent decades. Cells use various post-transcriptional regulatory mechanisms, such as alternative splicing, RNA localization, stability and non-coding RNAs, to temporally and coordinately influence the rate of protein synthesis. Today's gene expression research seeks to understand the dynamics of RNA regulation, with the ultimate goal of bridging the gap between transcriptional control and protein expression. RNA-binding proteins (RBPs) play a key role in posttranscriptional regulation of gene expression. RBPs can bind to RNA through an RNA recognition motif (RRM) or RNA-binding domain (RBD) in either the nucleus or the cytoplasm, depending on the type of RBP and the associated RNA sequence.

RNA-binding Protein Immunoprecipitation (RIP)

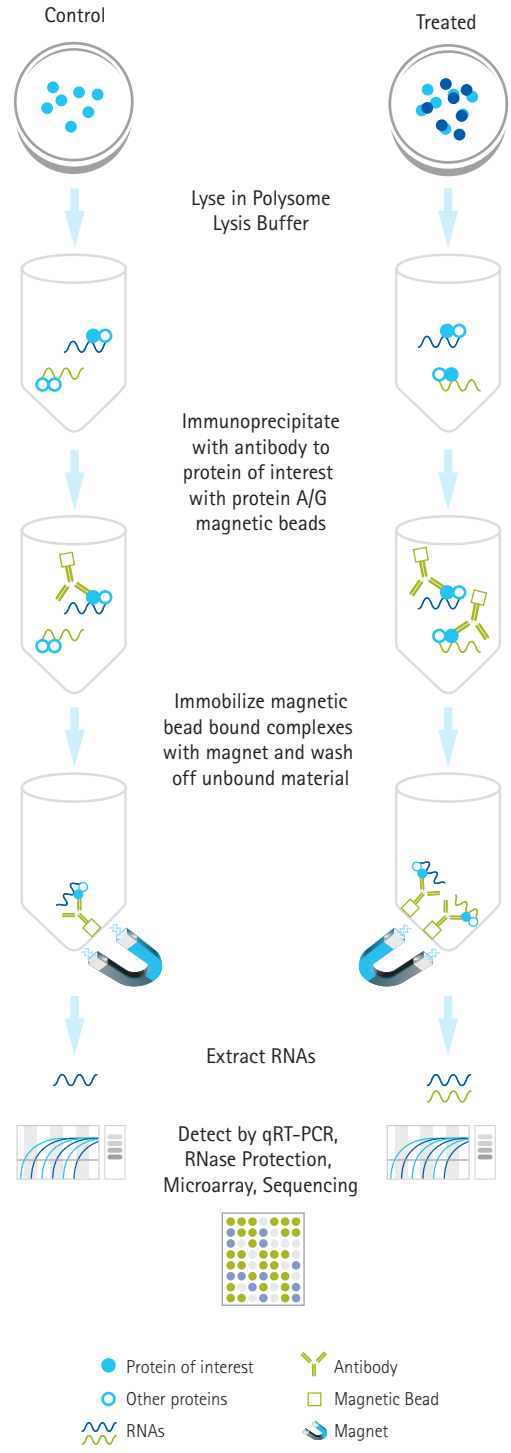
RIP can be viewed as the RNA analog of the more well-known ChIP application. RIP can be used to identify specific RNA molecules associated with specific nuclear or cytoplasmic binding proteins. RIP begins with immunoprecipitation of endogenous complexes of RNA-binding proteins and co-isolation of RNA species associated with the immunoprecipitated complex. After purification of these RNA species, they can be interrogated and identified as mRNAs or non-coding RNAs by a variety of applications including quantitative RT-PCR, microarray analysis (RIP-Chip) and high throughput sequencing (RIP-Seq).

Magna RIP™ and EZ-Magna RIP™ Immunoprecipitation Kits

- Protein A/G magnetic bead blend
- Compatible with an extensive line of RIPAb+™ validated antibodies (see page 28)
- A complete set of optimized reagents including RNAse inhibitors
- Essential positive and negative control antibodies, and qPCR primers
- Detailed protocols

Description	Catalogue No.
Magna RIP™ Kit, 12 reactions	17-700
EZ-Magna RIP™ Kit (with positive control antibody and primers, 12 reactions)	17-701

RIP Workflow



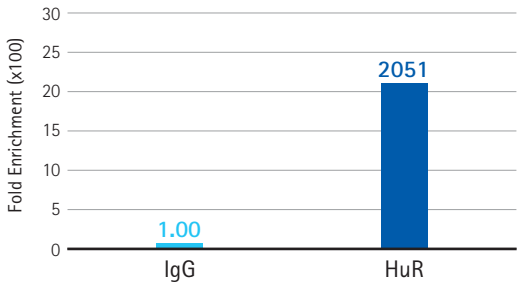
RIPAb+™ Antibody/Primer Sets

The RIPAb+™ kit includes a precision antibody, a negative control antibody to test specificity of the RIP reaction; plus control primers against a known enriched locus to help you validate your results.

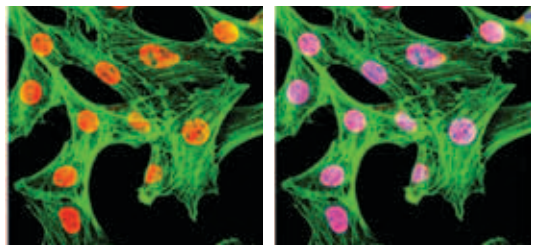
RIPAb+™ HuR

HuR stabilizes mRNAs, regulating gene expression, by binding to AU-rich sequences.

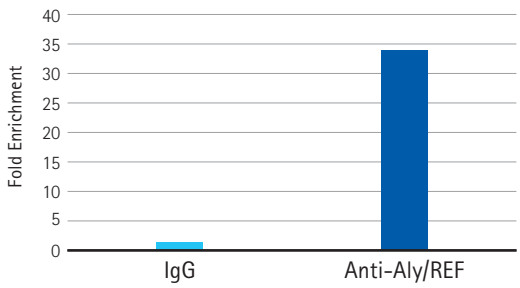
RIPAb+™ HuR antibody and the Magna RIP™ kit were used to enrich HuR:RNA complexes from HeLa cell extracts. Successful precipitation of HuR-associated RNA was verified by qPCR using RIP primers, ACTB (Catalogue No. CS203211).



Confocal IF analysis of HeLa, NIH 3T3 using anti-HuR (Red). Actin filamens have been labeled with AlexaFluor® 488 -Phalloidin (Green). Nucleus is stained with DAPI (Blue).



RIPAb+™ Aly/REF antibody and the Magna RIP™ kit were used to enrich Aly/REF:RNA complexes from Jurkat cell extracts. Successful precipitation of Aly/REF-associated RNA was verified by qPCR using RIP primers, DHFR-1 (Catalogue No. CS204401).



Description	Catalogue No.
RIPAb+™ Ago2	03-110
RIPAb+™ Aly/REF	03-120
RIPAb+™ AUF1	03-111
RIPAb+™ CUGBP1	03-104
RIPAb+™ CUGBP2	03-119
RIPAb+™ EED	03-196
RIPAb+™ EF1α	03-107
RIPAb+™ Fragile X Mental Retardation Protein	03-108
RIPAb+™ FXR1	03-176
RIPAb+™ FXR2	03-246
RIPAb+™ G3BP1	03-180
RIPAb+™ Gemin2	03-202
RIPAb+™ Gemin6	03-203
RIPAb+™ Hexim 1	03-177
RIPAb+™ Hexim 2	03-245
RIPAb+™ hnRNP C1/C2	03-205
RIPAb+™ hnRNP M1-M4	03-100
RIPAb+™ hnRNP U	03-206
RIPAb+™ hnRNPA1	03-204
RIPAb+™ hnRNPA1 (M9 Region)	03-181
RIPAb+™ HuR	03-102
RIPAb+™ IGF2 mRNA-binding protein 3	03-198
RIPAb+™ Lin28	03-105
RIPAb+™ LSM14A	03-184
RIPAb+™ Musashi 1	03-114
RIPAb+™ Musashi 2	03-115
RIPAb+™ p54nrb/NonO	03-113
RIPAb+™ PABPC1	03-101
RIPAb+™ pan Ago	03-248
RIPAb+™ Phospho-eIF4E (Ser209)	03-199
RIPAb+™ QKI-5	03-112
RIPAb+™ SMN	03-200
RIPAb+™ SNRNP70	03-103
RIPAb+™ SUZ12	03-179
RIPAb+™ Upf1	03-191

For a complete selection of RIPAb+™ Kits, please visit: **www.millipore.com/epigenetics**

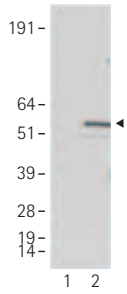
RNA ANALYSIS: RNA-BINDING PROTEIN ANTIBODIES

Description	Catalogue No.
Anti-4E-BP1, Rabbit Monoclonal	04-321
Anti-Ago1	07-599
Anti-Ago1, clone 6D8.2	04-083
Anti-Ago2	07-590
Anti-Ago2, clone 9E8.2	04-642
Anti-Ago4, clone 5F9.2	05-967
Anti-Ago Family	04-085
Anti-AIRE	09-456
Anti-AKAP 150	07-210
Anti-AKAP 95	06-417
Anti-AUF1	07-260
Anti-BRAF35, clone 4.21	05-641
Anti-CtBP-1	07-306
Anti-CUGBP1, clone 3B1	05-621
Anti-CuGBP2, clone 1H2	04-047
Anti-Dicer1, clone 5D12.2	04-721
Anti-Ebp1	07-397
Anti-EF1a, clone CBP-KK1	05-235
Anti-eIF4E CT, Rabbit Monoclonal	04-347
Anti-ESET/SetDB1	07-1568
Anti-hnRNP A0	07-504
Anti-hnRNP K, clone F45P9C7	04-088
Anti-hnRNP M1-M4, clone 1D8	05-620
Anti-HuR	07-1735
Anti-HuR	07-468
Anti-Iron Regulatory Protein 1	AB15506
Anti-Iron Regulatory Protein 2	AB15508
Anti-Lin28	07-1385
Anti-MBNL, clone 3A4	04-048
Anti-MDM2	07-575
Anti-Musashi	AB15648
Anti-Nova-1	07-637
Anti-Nucleolin	05-565
Anti-p68, clone PAb204	05-850
Anti-PABP, clone 10E10	04-1467
Anti-PABPC4, clone 6E1.2	MAB11015
Anti-PGC-1	AB3242
Anti-phospho eIF4E (Ser209)	07-823
Anti-phospho-eIF2Be (Ser539)	07-822
Anti-phospho-eIF-2a (Ser51)	07-760
Anti-phospho-eIF4G (Ser1108)	07-824
Anti-phospho-hnRNP A0 (Ser84)	07-566
Anti-PUM2, clone 1E10	MAB10104

For a complete selection of antibodies for RNA analysis, please visit: **www.millipore.com/epigenetics**

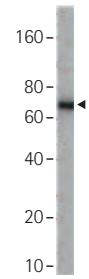
Pre-mRNA Processing: Nova-1

Nova-1 regulates alternative processing of neuronal pre-mRNAs. Spinal cord lysate (20 µg) from Nova-1 knockout (lane 1) and wild type (lane 2) mice were analyzed by Western blot and probed with anti-Nova-1 (07-637, 1:1000). Nova-1 is indicated by the arrow.



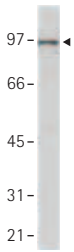
mRNA Stability: Poly-A Binding Protein 1 (PABP1)

Poly-A binding protein 1 (PABP1) binds to the poly A tail of mRNA transcripts to regulate mRNA stability. Additionally, PABP1 binding is coupled to pre-mRNA processing, regulation of translation initiation, and the mRNA decay pathway. Here, PABP1 (indicated by arrow) was detected in HeLa cells by Western blotting, using the PABP1 antibody (04-1467) and HRP-goat-anti-mouse IgG.



ncRNA-mediated Gene Regulation: Ago1

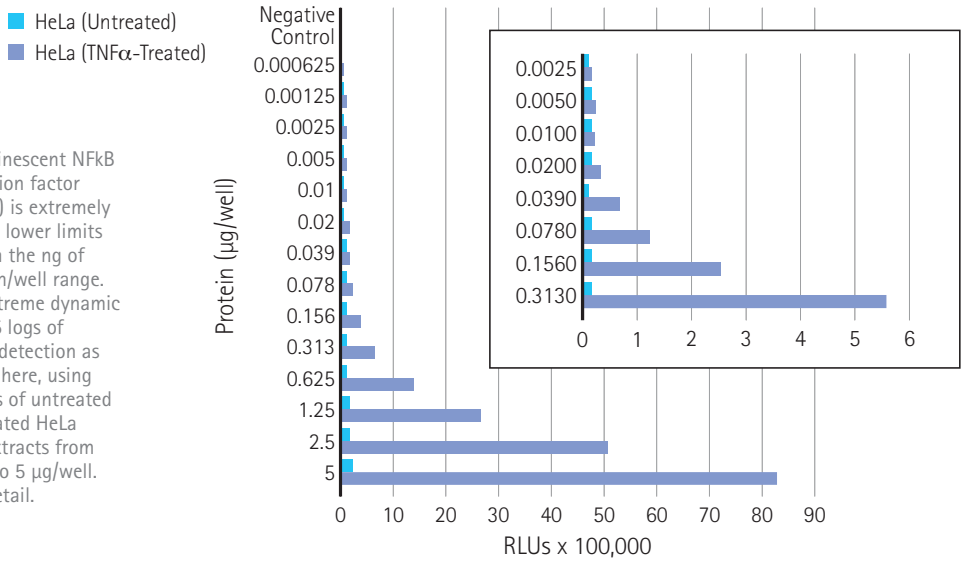
Ago1 (indicated by arrow) was detected in 20 µg of HeLa cell lysate by Western blotting, using the Ago1 antibody (07-599) and HRP-conjugated goat anti-rabbit IgG. ncRNA-mediated gene silencing (RNA interference, or RNAi) is catalyzed by the RNA-induced silencing complex (RISC). RISC is comprised of Argonaute (Ago) proteins and accessory RNAs, and mediates mRNA degradation by complementary small double-stranded RNAs.



Analyze DNA-protein interactions without messy radioactivity or running time-consuming gels. The EZ-TFA™ transcription factor assays provide a fast, sensitive method to detect specific DNA binding activity in whole cell or nuclear extracts. The assay enables high-throughput sample analysis with greater sensitivity than conventional electrophoretic mobility shift assays. Choose from universal kits that allow you to design an assay for your target of interest or one of our pre-configured target-specific assays.

NFκB p65 EZ-TFA™ Transcription Factor Assay (Chemiluminescent)

The transcription factor NFκB (Nuclear Factor kappa B) is involved in the expression and regulation of a number of important cellular and physiological processes such as growth, development, apoptosis, immune and inflammatory responses. The p50/p65 heterodimer of NFκB is the most abundant in cells. The NFκB EZ-TFA™ p50 and p65 assays are powerful tools for measuring active NFκB in nuclear extracts.



The chemiluminescent NFkB p65 transcription factor assay (70-620) is extremely sensitive, with lower limits of detection in the ng of nuclear protein/well range. The assay's extreme dynamic range covers 5 logs of magnitude of detection as demonstrated here, using serial dilutions of untreated and TNFα-treated HeLa cell nuclear extracts from 0.000625 µg to 5 µg/well. Inset shows detail.

Description	Catalogue No.
Universal EZ-TFA™, Colorimetric	70-500/501
Universal EZ-TFA™, Chemiluminescent	70-600/601
EZ-TFA™ NFκB p65, Colorimetric/Chemiluminescent	70-520/620
EZ-TFA™ NFκB Family, Colorimetric/Chemiluminescent	70-560/660
EZ-TFA™ NFκB p50/p65, Colorimetric/Chemiluminescent	70-510/610
EZ-TFA™ NFκB p50, Colorimetric/Chemiluminescent	70-515/615

Gene transfer, resulting in either gain of function or loss of function, is a key technique for studying the effects of protein function on specific cellular pathways. Since the establishment of RNAi as an effective gene silencing method, researchers have used small interfering RNAs (siRNAs), either introduced into cells or transcribed from integrated DNA sequences, as tools to study how loss of function of target genes affects cellular outcomes.

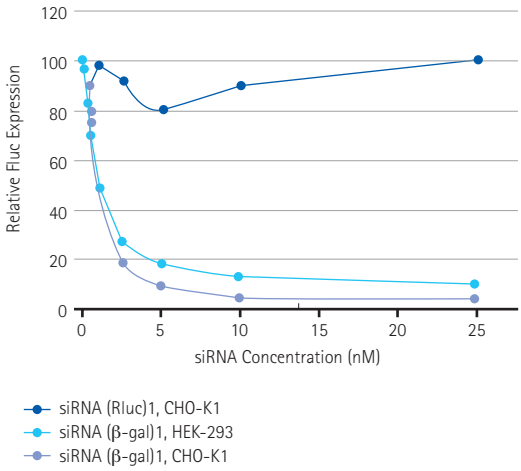
RiboJuice™ siRNA Transfection Reagent

Features

- Suitable for both stable and transient transfections with siRNA
- Minimal cellular toxicity
- Compatible with both serum-containg and serum-free media

Benefits

- One reagent for a variety of applications
- Leads to higher siRNA-mediated suppression of protein expression
- Simplifies protocol by eliminating media changes



Concentration dependence and specificity of siRNA (β-gal)-mediated suppression. CHO-K1 and HEK-293 cells were transfected after 24 h with two mixtures. The first mixture contained 1 µL GeneJuice® Transfection Reagent, 0.25 µg pTriEx™-2 (β-gal), and 0.025 µg pTriEx™-2(Fluc). The second mixture contained 3 µL RiboJuice™ siRNA Transfection Reagent and various concentrations of the indicated siRNAs. As a control, 3 µL RiboJuice™ Reagent was also used without any siRNA. Total volume per well was 300 µL. Lysed cells were assayed for reporter activity after 24 h. The siRNA(Rluc)1 sequence was AAACAUGCAGAAAAUGCUGUUUU.

Description	Catalogue No.
RiboJuice™ siRNA Transfection Reagent	71115-3
RiboJuice™ siRNA Transfection Reagent	71115-4
Fast-Trap® Adenovirus Purification and Concentration Kit	FTAV00003
Fast-Trap® Lentivirus Purification and Concentration Kit	FTLV00003
Fast-Trap® Adeno-Associated Virus (AAV) Purification and Concentration Kit	FTAA00003

DNA Structure, Damage and Repair

DNA is organized into chromosomes to allow packaging into the nucleus, but also to enable cells to differentiate, divide, and endure environmental stresses, while protecting its valuable genetic information. DNA structure and organization enables the cell to divide DNA evenly between mother and daughter cells, avoiding aneuploidy, unnecessary gene duplication or deletion. Chromosomal instability is a hallmark of many cancers, and is seen as either a cause or a symptom of the unchecked proliferation exhibited by tumor cells.

By tightly regulating chromosome duplication, movement and separation during the cell cycle, the cell protects the genome from damage. However, a certain amount of damage, either due to DNA replication errors, age-shortened telomeres, or environmental causes, is unavoidable. To repair DNA damage, or to minimize its tumor-causing potential, cells rely on a multi-component damage detection and repair system.

Studying the mechanisms by which cells control changes in DNA structure and respond to DNA damage will help to elucidate the factors that cause aging, cellular degeneration, cancer, and death.

TELOMERE MAINTENANCE: KITS AND ANTIBODIES

Located at the ends of eukaryotic chromosomes, telomeres consist of thousands of DNA repeats. Telomeres protect chromosome ends, limiting fusion, rearrangement and translocation. In somatic cells, telomere length is progressively shortened with each cell division, because DNA polymerase cannot synthesize the 5' end of the lagging strand. Telomerase is a ribonucleoprotein that synthesizes telomeric repeats using its RNA component as a template. Telomerase expression and telomere length stabilization are linked to extension of cell life span and tumor suppression.

TRAPeze® Telomerase Detection Kit

EMD Millipore provides a broad range of products for assaying telomerase activity. TRAPeze® telomerase detection kits are rapid, quantitative, *in vitro* assays for detecting activity. The original kit permits detection via PCR and gel electrophoresis. TRAPeze® telomerase detection kits are also available in colorimetric and fluorimetric formats as the TRAPeze® ELISA and TRAPeze® XL kits, incorporating biotinylated and fluorescent primers respectively.

1 2 3

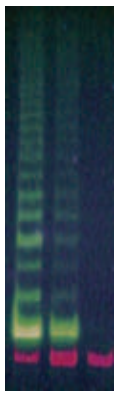
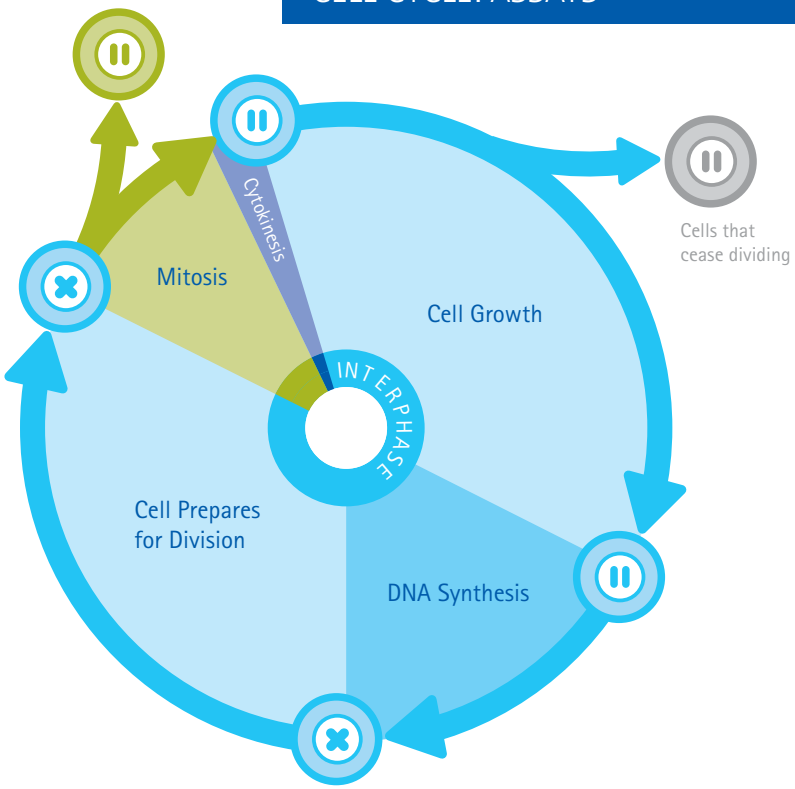


Image demonstrates the direct fluorescence imaging of the TRAPeze® XL reaction of three specimens – telomerase positive lanes 1 and 2, and telomerase negative lane 3.

Description	Catalogue No.
TRAPeze® Telomerase Detection Kit	S7700
TRAPeze® XL Telomerase Detection Kit	S7707
TRAPeze® ELISA Telomerase Detection Kit	S7750
TRAPeze® RT Telomerase Detection Kit	S7710
TRAPeze® Positive Control Cell Pellet	S7701
Anti-TRF1, clone BED5 57-6	04-638
Anti-TRF2, clone 4A794	05-521
Telomerase Inhibitor III, Sodium Salt	581004
Telomerase Inhibitor VI, Sodium Salt	581006

CELL CYCLE: ASSAYS



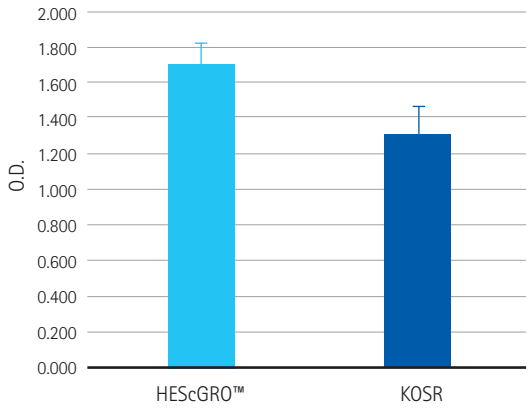
Cell cycle, or the process of cell growth and duplication, is the regulatory point for proliferation and development of multicellular organisms. Nuclear signaling controls most checkpoints of the cell cycle, and is in turn regulated by chromatin structure.

BrdU Cell Proliferation Kit

- Non-radioactive
- Colorimetric detection

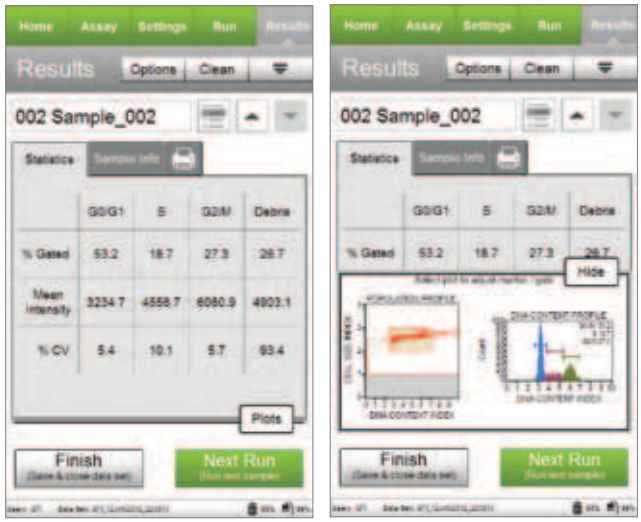
The BrdU cell proliferation kit (2750) was used to measure proliferation of H9 human embryonic stem cells in HEScGRO™ and KOSR media, after cells were enzymatically expanded for 12 passages. Increased BrdU incorporation indicated faster cell proliferation in HEScGRO™ medium.

Description	Catalogue No.
BrdU Cell Proliferation Kit	2750



Muse™ Cell Cycle Assay

The Muse™ cell cycle assay allows for the facile, rapid, and quantitative measurements of percentage of cells in the G0/G1, S, and G2/M phases of cell cycle on the Muse™ cell analyzer, a small-footprint instrument with simple sample prep, miniaturized optics and touchscreen analysis. The assay simplifies an analysis that has traditionally required complicated instrumentation and provides information on cell cycle distribution on the benchtop. The Muse™ cell cycle assay exploits the differential staining of cells by propidium iodide, depending on DNA content (which changes depending on the phase of the cell cycle). By simply adding the cell cycle assay reagent to fixed cells for 30 min and following the instructions on the intuitive touchscreen, any user can achieve accurate, precise cell cycle distributions for a wide variety of cell lines.

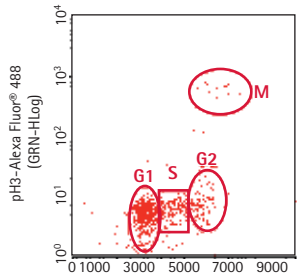


Cell cycle distribution in Jurkat cells, calculated using the Muse™ cell cycle assay and using the Muse™ cell analyzer. The screen at left shows numerical values and statistics, while the screen at right shows the dotplots and histograms of the same data.

Description	Catalogue No.
Muse™ Cell Cycle Assay	MCH100106

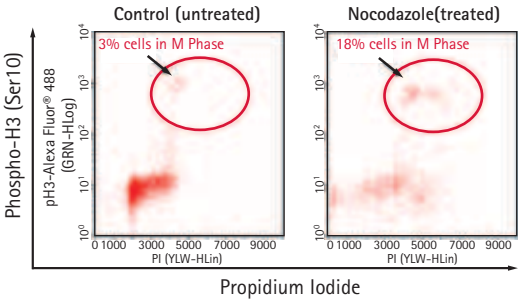
FlowCellect™ Bivariate Cell Cycle Kit for G2/M Flow Cytometry Analysis

Investigate the G2/M phase transition with this convenient, accurate flow cytometry kit. The phosphorylation of histone H3 at Ser10 correlates with the G2 to M phase transition and is a prerequisite for chromatin condensation at mitosis. Therefore, phospho-Histone H3 (Ser10) is a reliable, specific marker of M-phase cells.



Cell Cycle Phases:
G1 = 57%
S = 19%
G2 = 15%
M = 3%

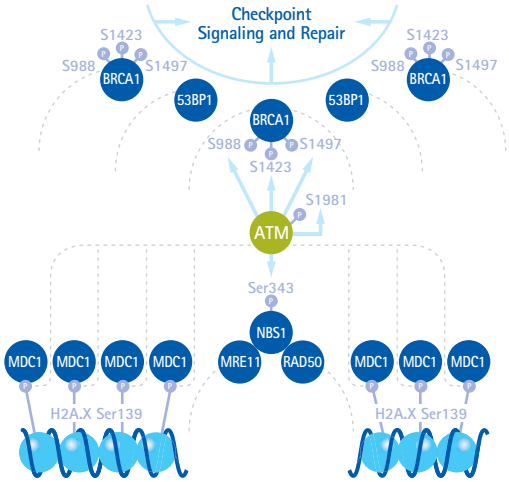
Discrimination between G2 and M phase cells by measuring the phosphorylation of Histone 3 on Ser10. Histone 3 is constitutively phosphorylated at Ser10 during metaphase.



Nocodazole treated cells to determine cells in M-phase. Cells were either treated with 100 mM Nocodazole (test sample) or left untreated (control) overnight at 37 ˚C. By plotting the phosphorylation of H3 at Ser10 versus DNA content, an increase in the proportion of G2/M cells was observed indicating that mitotic cells have accumulated after treatment. Approximately 2% of cells reside in M phase under normal conditions in Jurkat cells, but when treated cell population increases to 18%.

Description	Catalogue No.
FlowCellect™ Bivariate Cell Cycle Kit for G2/M Flow Cytometry Analysis	FCCH025103
FlowCellect™ Bivariate Cell Cycle Kit for DNA Replication Analysis	FCCH025102

Response to DNA damage is initiated by recognition of double-strand breaks by ATM kinase and the Nbs1/ Mre11/ Rad50 complex. Phospho-H2A.X binds MDC1 to help recruit other damage-response proteins. ATM phosphorylates BRCA1, a key effector of checkpoint/ repair signaling. Other proteins localize the signaling to the damage site, such as 53BP1, which recruits p53. p53 causes the cell cycle to pause, providing repair machinery the opportunity to fix the damage. If the damage is too severe, p53 signals the cell to undergo apoptosis.

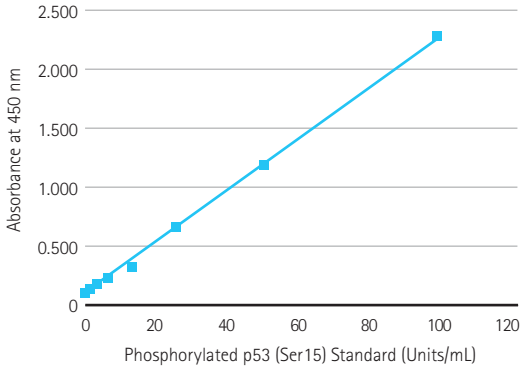


In this example to a cell's response to DNA damage, ATM kinase responds to H2A.X phosphorylation by phosphorylating multiple targets and coordinating assembly of repair complexes.

p53 STAR ELISA Kit

In response to DNA damage, p53 induces gene expression, such as for the Cdk inhibitor p21, which, in cooperation with p19ARF, causes cell cycle arrest. Inactivation or loss of p53 is associated with deregulation of the cell cycle and DNA replication, inefficient DNA repair, and the development of various human cancers. The p53 STAR (Signal Transduction Assay Reaction) ELISA is a fast, sensitive method to detect activated p53.

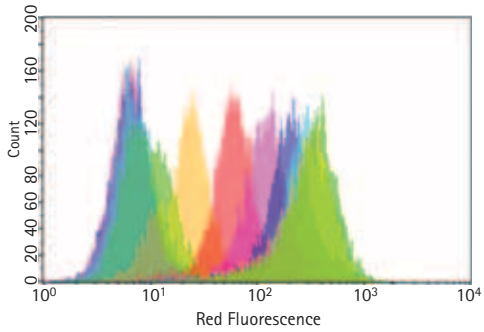
Description	Catalogue No.
Phospho-p53 (Ser15) STAR ELISA Kit	17-475
Anti-Chk1	04-207
Anti-Plk1	05-844
Anti-Wee1	06-972



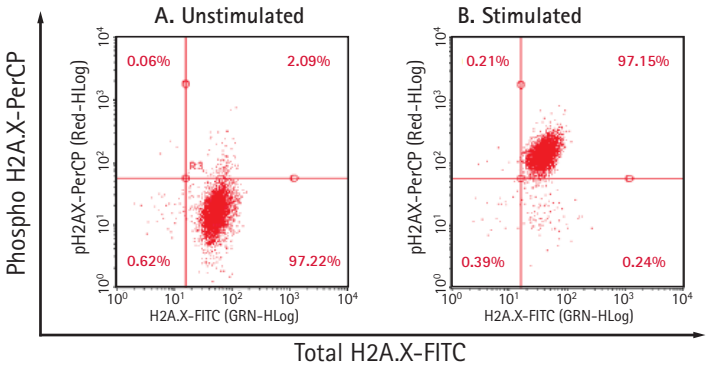
Typical p53 Standard Curve. 100 µL of progressive 2-fold dilutions of the p53 standard included in the kit and run as described in the assay instructions.

FlowCellect™ DNA Damage Histone H2A.X Dual Detection Kit

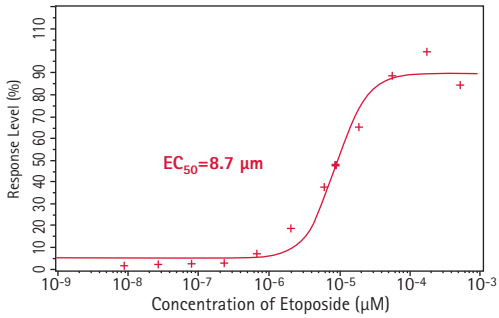
Phosphorylated H2A.X (Ser139) is the key component of the signal transduction pathways that are mobilized during DNA damage. The FlowCellect™ DNA Damage Histone H2A.X Dual Detection Kit uses pairs of total and phospho-specific antibodies for multicolor flow cytometry analysis. Simultaneous analysis of total and phosphorylated H2A.X will provide an accurate method to investigate levels of phospho-specific H2A.X and the degree of DNA damage.



InCyte™ software was used to generate overlaid histograms showing that increasing doses of etoposide resulted in increasing red fluorescence and increasing phosphorylation of H2A.X.



Dual Parameter Analysis of Total and Phospho Histone H2A.X on HeLa Cells. In untreated HeLa cells stained with both anti-phospho-Histone H2A.X-PerCP and Anti-Histone H2A.X-FITC (A), 97.2% of cells show positive signal for total H2A.X but no Histone H2A.X activation via phosphorylation. However, once HeLa cells were treated with 100 µM etoposide, 97.15% of the cells became positive for both total and phospho-H2A.X, confirming target specificity of the phosphorylation event (B). Only 2.09% of untreated cells were double positive (A).



Using the curve-fitting functions of InCyte™ software, an EC_{50} of 8.7 µM was calculated for etoposide's effect on HeLa cells under the conditions of this experiment.

Description	Catalogue No.
H2A.X Phosphorylation Assay Kit, Flow Cytometry	17-344
FlowCellect™ DNA Damage Histone H2A.X Dual Detection Kit	FCCS025153
FlowCellect™ Histone H2A.X Phosphorylation Assay Kit	FCCS100182
H2A.X (Ser139) Dual Detect CELISA Assay Kit (Fluorogenic Detection)	17-720
FlowCellect™ Cell Cycle Checkpoint H2A.X DNA Damage Kit	FCCH025142

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