MILLIPORE™

Product Selection Guide

Epigenetics & Gene Regulation

Antibodies, Proteins, Kits, and Assays

upstate | CHEMICON

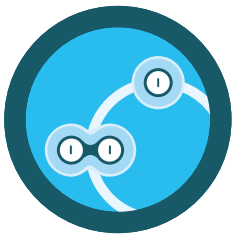
THE EXPERTISE OF UPSTATE® AND CHEMICON®
IS NOW A PART OF MILLIPORE

Platforms and Technologies

As a tools provider and partner in research, Millipore is committed to the advancement of life science research and therapeutic development. This guide includes a number of new products for target identification, pathway detection and profiling. These products provide proven solutions for a range of applications and are backed by expert technical support.

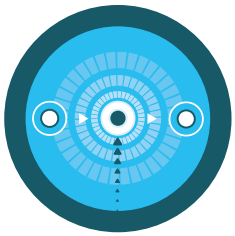
Antibodies and Immunoassays

Millipore offers an extensive, focused portfolio of antibodies and immunoassays. With the expertise of Upstate® and Chemicon®, Millipore provides validated products with breadth and depth in major research areas backed by excellent service and support.



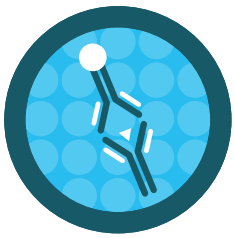
Cell Based Assays and High Content Analysis

Millipore offers a significant portfolio of live cell, whole-cell and cell-based activity assays and reporter systems for direct and indirect detection. These technologies facilitate protein target validation, identify cellular pathways and determine mechanism of action for lead optimization environments. Millipore also offers an array of assays for high-content multi-parametric analysis; enabling identification of cellular responses and events under user-defined conditions.



Flow Cytometry Assays and Systems

Flow cytometry is an essential tool for in-depth cell analysis, with the capacity to simultaneously measure multiple parameters on individual cells. Guava® flow cytometers provide direct, precise measurement via microcapillary technology that translates into smaller samples, less reagents, and minimal waste. Millipore also offers FlowCellect™ reagents and kits that are optimized for guava systems and compatible with traditional core lab environments, along with application-specific analysis software modules, to provide a complete solution for flow cytometry.



MILLIPLEX® MAP Multiplex Assays

MILLIPLEX MAP assays offer the broadest selection of multiplex kits and reagents in a wide variety of therapeutic areas, measuring multiple biomarkers using a small sample size. Compared to conventional methods, such as ELISAs and Western blots, MILLIPLEX MAP enables the simultaneous detection of multiple soluble or intracellular biomarkers. Using the Luminex® xMAP® bead-based technology, Millipore's flexible and customizable assays are exhaustively tested and qualified for sensitivity, specificity, reproducibility and wide dynamic range.

Introduction

Epigenetics describes heritable changes in gene expression caused by non-genetic mechanisms instead of by alterations in DNA sequence. Epigenetic changes can persist throughout a cell's life and be passed on to multiple generations. Epigenetic mechanisms are classically considered to be centered in the nucleus. However, epigenetic modifications can occur in response to environmental signals such as hormones, nutrients, stress, and cellular damage, pointing to the involvement of cytoplasmic and extracellular factors in epigenetic regulation. Epigenetic regulation enriches DNA-based information, allowing a cell to vary its response across diverse biological and environmental contexts. Epigenetic changes can effect transcriptional and post-transcriptional regulation via mechanisms such as:

- 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 using sequences that are virtually identical from cell to cell. Understanding epigenetic mechanisms of gene regulation impacts diverse areas of research, from agriculture to human health. Currently, researchers apply epigenetics research tools to a variety of fields of study, including:

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

Over the past decade, the study of epigenetics has shifted from basic mechanisms to their effect on development and disease. Throughout this time, Millipore has been dedicated to developing and refining technologies for the study of epigenetic phenomena. With a comprehensive portfolio, including the former Upstate® and Chemicon® brands of reagents and antibodies, researchers can count on dependable, high quality reagents and expert support for their studies of gene regulation.

www.millipore.com/epigenetics

TABLE OF CONTENTS

COUNT ON MILLIPORE FOR KITS, ANTIBODIES, PROTEINS AND REAGENTS FOR:

- Chromatin assembly
- Chromatin immunoprecipation (ChIP)
- RNA-binding protein immunoprecipitation (RIP)
- Analysis of histone modifications (acetylation, methylation, phosphorylation)
- Bisulfite conversion of methylated DNA
- Methylated DNA enrichment
- Methlyation-specific PCR assays
- Transcription factor binding assays
- Genome-wide epigenetic analysis

Chromatin Analysis

Chromatin is the complex of genomic DNA and associated proteins in the nucleus. This higher ordered structure of DNA allows cells to package their DNA, provides a scaffold for cell division, and enables control of gene expression. Chromatin structure, bound by a dynamic repertoire of proteins, alternates between condensed heterochromatin and extended euchromatin.

Modifications of chromatin structure and the interplay of histones and non-histone proteins play a direct role in epigenetic regulation. Histones, a major class of chromatin proteins, 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. Millipore offers a range of kits, assays, recombinant proteins, and antibodies to enable the study of chromatin and histones.

Chromatin Proteins, Assembly, and Remodeling

Chromatin is the natural state of DNA in the nucleus, and 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

Millipore's proteins for *in vitro* chromatin assembly include histone chaperone NAP-1, which regulates chromatin fluidity, and Acf1/ISWI, a chromatin remodeling complex required for replication through heterochromatin. 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 for analyzing the chromatin assembly process.

CHROMATIN HISTONES AND RECOMBINANT HISTONES

Description	Catalogue No.
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

CHROMATIN ASSEMBLY ANTIBODIES

Proteins involved in assembling chromatin also regulate its remodeling, thereby affecting transcription. Study the details of the process with antibodies against histone modifying enzymes, helicases, and more.

Description	Catalogue No.
Anti-CHD9	09-090
Anti-hSNF2H	07-624
Anti-Mi-2	06-878
Anti-Mi-2b (CHD4)	06-1306
Anti-SNF2β/BRG1	07-478

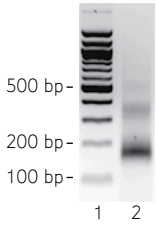
Chromatin Immunoprecipitation (ChIP)

ChIP is a powerful technique for studying protein-DNA complexes. Specific antibodies enrich for regions of chromatin (sheared to a manageable size and harvested from cells) that contain the protein of interest, and various detection methods (quantitative PCR (qPCR), microarray) are employed to detect specific DNA sequences within the enriched chromatin. Antibodies enrich for chromatin regions that contain the protein of interest. Various detection methods identify specific DNA sequences within the enriched chromatin. Since Upstate, now part of Millipore, launched the first ChIP kits in the 1990s, Millipore has introduced magnetic beads, the use of nuclear extracts, control antibodies and primers, quantitative and multiplex PCR capabilities, and a faster (1-day) protocol. Today, our ChIP kits are even more advanced, providing:

- Optimized, specialized protocols
- Improved sample prep
- Alternate detection methods
- Automation compatibility
- Genome-wide analysis
- ChIP-validated antibodies

EZ-Zyme™ Chromatin Preparation Kit (17-375)

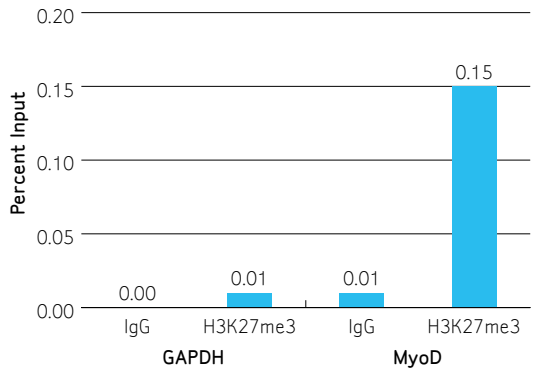
For a gentle alternative to sonication, use the EZ-Zyme chromatin preparation kit to fragment chromatin DNA. This kit renders chromatin immunoprecipitation more efficient due to mild digestion conditions, and is compatible with non-crosslinked ChIP (native ChIP), enabling analysis of histone modifications not only in cultured cells, but also in freshly dissected and frozen tissues.



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.

EZ Magna ChIP Chromatin Immunoprecipitation Kits (17-408 and 17-409)

Magna ChIP kits make it possible to complete a ChIP experiment in a single day, from cell harvest to PCR results. Suitable for high throughput applications and available with either protein A or protein G, Magna ChIP kits permit easy optimization for any target antibody. EZ-Magna ChIP kits also contain essential positive and negative controls to ensure high quality results.



Sonicated chromatin prepared from 2 x 10^6 HeLa cells was subjected to chromatin immunoprecipitation using 4 µg purified ChIPAb+ anti-Trimethyl-Histone H3 (Lys27) (17-622) or normal rabbit IgG and the Magna ChIP A kit (17-610). Because H3K27 methylation is associated with gene silencing, it was expected that the silent MyoD promoter, compared to the active GAPDH promoter, would be enriched by this immunoprecipitation. Successful enrichment of trimethyl-Histone H3 (Lys27) associated DNA fragments was verified by qPCR using ChIP Primers GAPDH (22-004) flanking the human GAPDH promoter and primers targeting the promoter of human MyoD.

Magnetic Racks for ChIP Assays

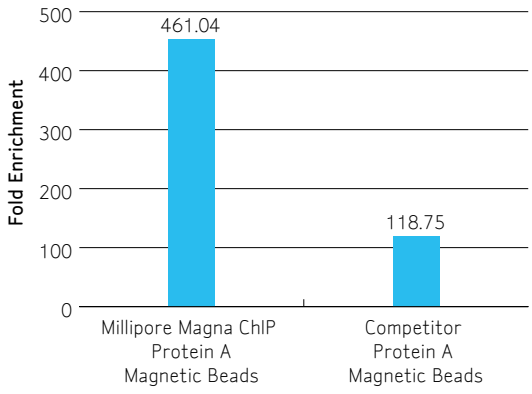
Choose one of our magnetic racks for Magna ChIP assays: the classic Magna GrIP™ rack, or the new, extra-strong, contoured PureProteome® magnetic stands, which are ideal for large scale ChIP.

Description	Catalogue No.
Magna GrIP Rack (8-well)	20-400
PureProteome Magnetic Stand (8-well, removable magnet)	LSKMAGS08
PureProteome Magnetic Stand (15 mL)	LSKMAGS15

Magna ChIP Protein A Magnetic Beads* (16-661)

Magna ChIP magnetic beads are optimized specifically for ChIP applications and are a rapid, reproducible, and efficient reagent for collecting immunocomplexes in ChIP ssays. Unlike conventional agarose beads, Magna ChIP magnetic beads are isolated in seconds and significantly reduce the handling time and mechanical stress on target immunocomplexes.

***COMING SOON**: Magna ChIP protein G beads and Magna ChIP protein A+G beads! Contact us for details.



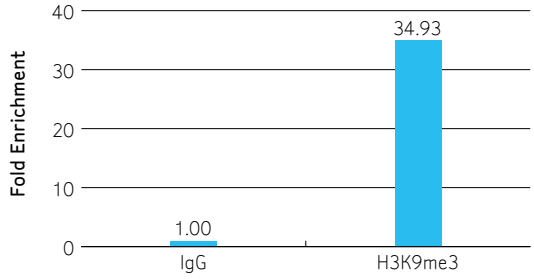
Enrichment of GAPDH promoter DNA by Magna ChIP Protein A Magnetic Beads vs. a leading competitor's magnetic beads in identical ChIP assays using ChIPAb+ Acetyl H3 antibody (17-615) and control rabbit IgG. GAPDH is constitutively active and its promoter is expected to be associated with acetylated Histone H3. Millipore's beads resulted in significantly greater change in PCR threshold values (ΔC_T not shown) and greater fold enrichment of GAPDH promoter DNA.

A GROWING SELECTION OF CHIP-QUALIFIED ANTIBODIES

Millipore also offers a wide selection of ChIP-qualified antibodies against modified and unmodified histones, transcription factors, and other key chromatin associated proteins. Search our complete list of antibodies for ChIP by visiting **www.millipore.com/epigenetics.**

ChIPAb+ Antibody/Primer Sets – Validated and Lot-Tested for Chromatin Immunoprecipitation

Antibody recognition in the context of chromatin can differ from other immunoassays. Avoid ChIP failure due to poor antibody performance by using ChIPAb+ antibodies, each lot of which is individually validated and tested for ChIP. Even when using a ChIP-grade antibody, detection conditions, including PCR primer quality, might affect your analysis. Each ChIPAb+ antibody set includes control primers (every lot tested by qPCR) to validate your ChIP results. The ChIPAb+ set also includes a negative control antibody to confirm specificity of the reaction.



ChIPAb+ Trimethyl Histone H3 (Lys9) (17-625): Sonicated chromatin from NIH3T3 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+ CREB	17-600
ChIPAb+ EZH2, clone AC22	17-662
ChIPAb+ HDAC1	17-608
ChIPAb+ p53	17-613
ChIPAb+ REST	17-641
ChIPAb+ RNA Pol II, purified	17-620
ChIPAb+ Sox-2, clone 6F1.2	17-656
ChIPAb+ Histone H2B	17-10054
ChIPAb+ Dimethyl-Histone H3 (Lys9)	17-681
ChIPAb+ Acetyl-Histone H4 (Lys5)	17-10045
ChIPAb+ Monomethyl-Histone H4 (Lys20)	17-651

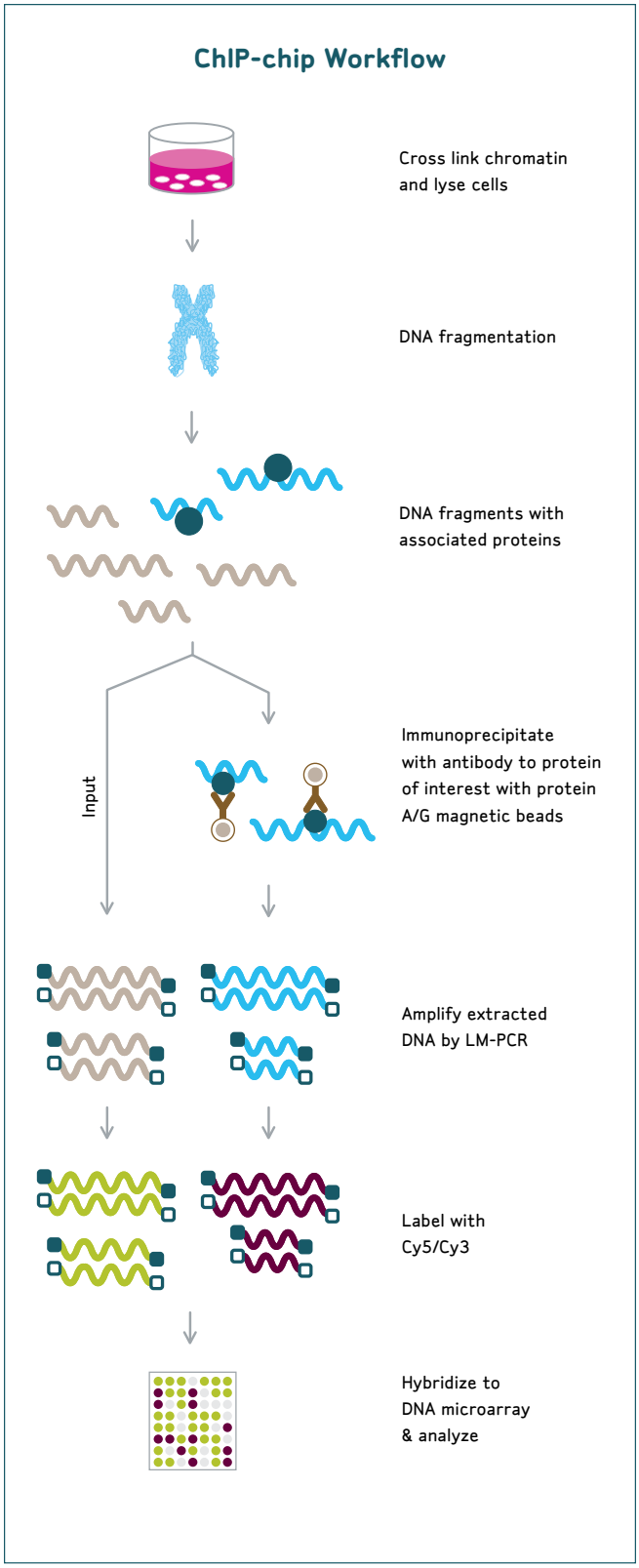
Genome-wide Chromatin Immunoprecipitation

Genome-wide analysis of ChIP-isolated DNA, either by microarray hybridization (ChIP-chip) or by sequencing (ChIP-seq), reveals how regulatory and structural proteins bind and interact across a genome. ChIP-chip can be performed using promoter or genomic tiling arrays. For ChIP-seq, ChIP fragments are analyzed using a genome sequencer. Although proteins with many binding sites can require deep, costly sequencing runs, advances in high resolution sequencing are lowering the cost of this high content, rapid analysis method.

While our Magna ChIP²™ kits are specially designed for ChIP-chip, all of Millipore's standard ChIP and Magna ChIP kits are appropriate for isolating enriched DNA for genome-wide ChIP-chip or ChIP-seq.

Magna ChIP² Chromatin Immunoprecipitation 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 the success, sensitivity and reproducibility using either Agilent® or user-provided DNA microarrays.



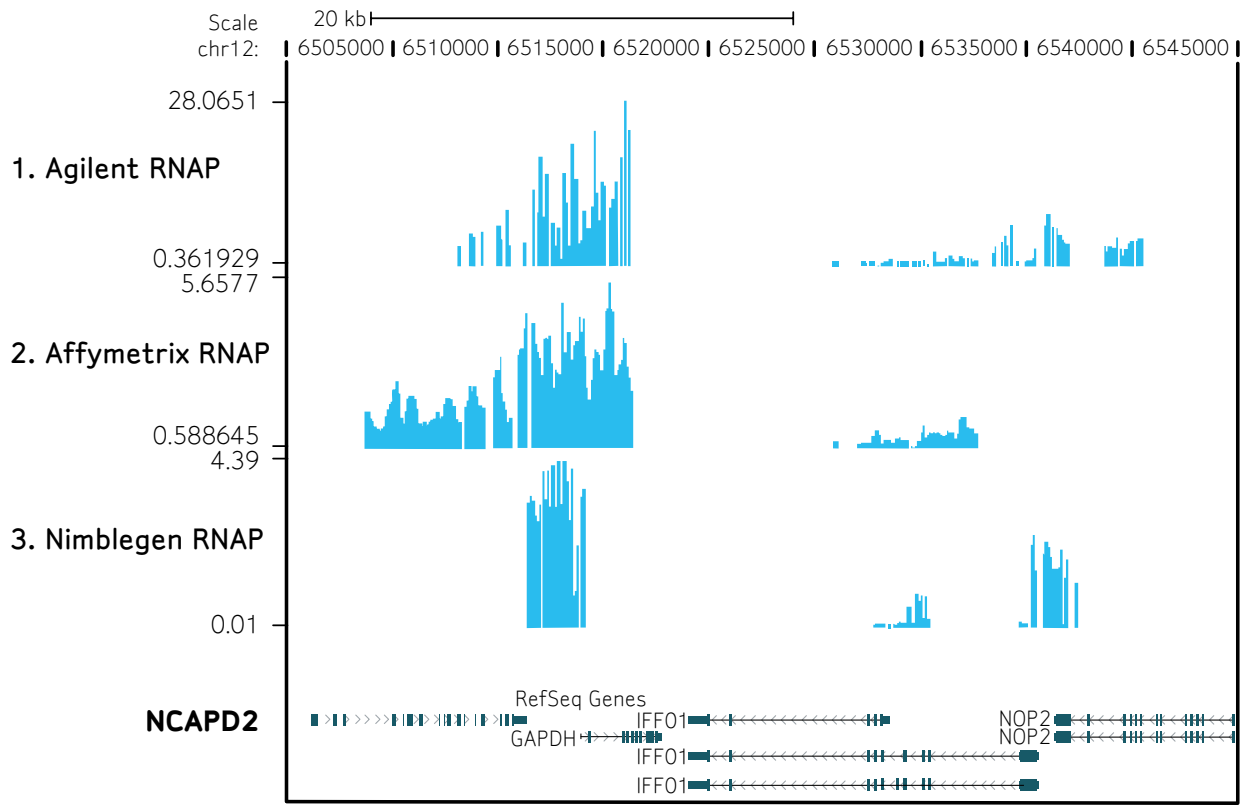
Magna ChIP² Universal Kits

Perform ChIP-chip analysis on virtually any type of microarray with the universal Magna ChIP² kits. The 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.

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 Millipore Magna ChIP² kit.

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

Description	Catalogue No.
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

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

Modified Histones

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. Millipore offers over 200 validated antibodies, recombinant proteins, and kits to analyze histone modifications.

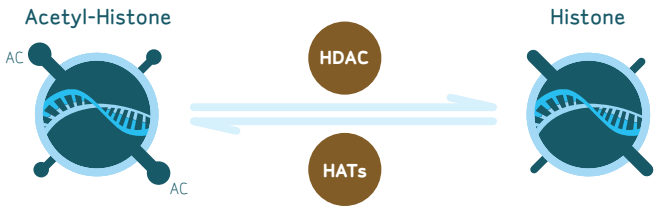
Availability of Antibodies to Histone Modifications

	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	K123		
Citrulline					R3

Table 1. Some of the specific histone modifications detected by 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 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.

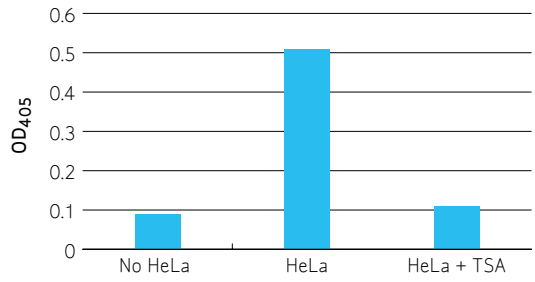
Acetylation

Histone acetylases (HATs) and deacetylases (HDACs) are key regulators of gene expression and function. Transcription activation complexes contain HATs, which, by acetylating histone lysines, open chromatin structure to permit transcription. HDACs remove acetyl groups, leading to decreased gene expression. Millipore's assay kits measure both acetylation and deacetylation activity, helping to elucidate the relationship between chromatin modification pathways and gene regulation.



HDAC Assay Kit, Colorimetric Detection (17-374)

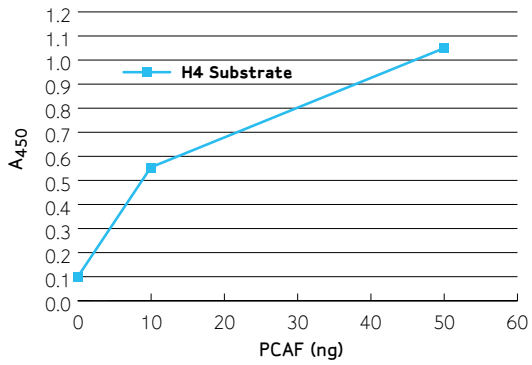
The colorimetric HDAC assay kit is a simple, two-step procedure designed for use in 96- or 384- multi-well plates. Alternatively, use the HDAC assay kits supporting fluorometric detection or radiometric detection.



Inhibition of HeLa Nuclear Extract by Trichostatin A
HeLa Nuclear Extract in the absence or presence of 1 µM Trichostatin A was assayed with the Colorimetric HDAC Assay Kit (17-374). Absorbance was detected at 405 nm.

HAT Assay Kit (17-289)

Measuring histone acetyltransferase activity is simple with this indirect ELISA kit containing everything needed to detect acetylated lysine residues on histones H3 or H4 via biotinylated histone peptides. Acetylated H3 and H4 peptides are included as positive controls, and can be used to generate standard curves for quantification.



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.
HAT Assay Kit	17-289
HDAC Assay Kit, Colorimetric	17-374
HDAC Assay Kit, Fluorometric	17-356
SIRT1 Deacetylase	17-370

Related Products

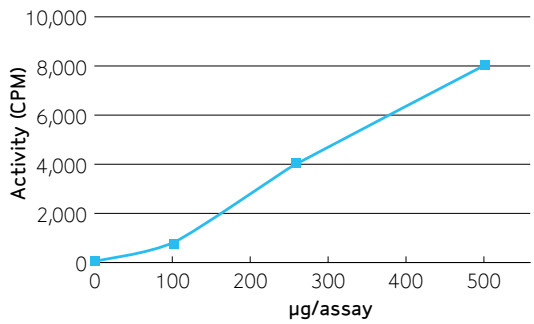
Description	Catalogue No.
HDAC1 Active recombinant protein	14-838
HDAC4 Active recombinant protein	14-828
HDAC7 Active recombinant protein	14-832
HDAC8 Active recombinant protein	14-609
SIRT1 Deacetylase	17-370

A GROWING SELECTION OF ANTIBODIES AND KITS FOR HISTONE MODIFICATION

We have a large selection of antibodies and kits for most Histone Modification studies - for complete listing, visit **www.millipore.com/epigenetics.**

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.



PRMT1 Histone Methyltransferase Assay:
100-500 ng of active PRMT1 (14-474) methylated core histones *in vitro*, using the Histone Methyltransferase Assay Reagent Kit (17-330). Assay background was subtracted from the actual counts to yield the results.

Description	Catalogue No.
Histone Methyltransferase Assay Reagent Kit	17-330
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

Related Products

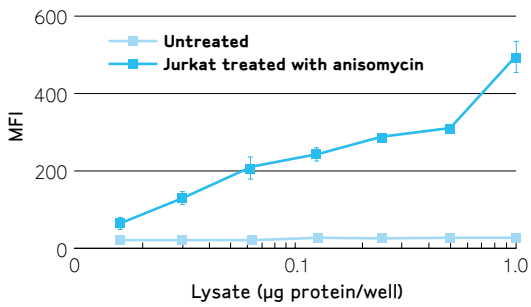
Description	Catalogue No.
CARM1, active	14-575
Anti-LSD1	09-058
Anti-JMJD3	07-1533
PRMT1, active	14-474
PR-SET7, active	14-539
SET9, active	14-469

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 either promote opening or closing of chromatin structure. Kinases with histone substrates include JAK2 tyrosine kinase and AMPK serine/threonine kinase.

H2A.X Phosphorylation Multiplex Assay Kit and MAPmates™ (46-692)

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.

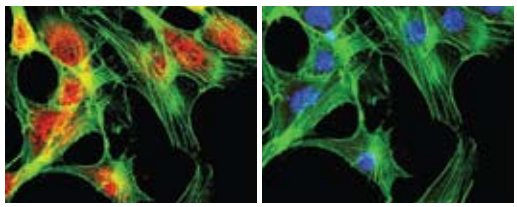


MILLIPLEX MAP detection of changes in phosphorylation of Histone H2A.X (Ser139) in Jurkat cells stimulated with or without 25 mM anisomycin. The Median Fluorescent Intensity (MFI) was measured using the Luminex Instrument.

Description	Catalogue No.
H2A.X Phosphorylation Assay Kit (Flow cytometry)	17-344
H2A.X Phosphorylation/DNA Damage Assay	HCS224
H2A.X Phosphorylation and p53 DNA Damage Assay	HCS225
Human Phospho-Histone H2A.X MAPmates™	46-692

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.

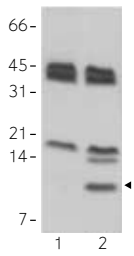


Confocal IF analysis of NIH 3T3 cells using anti-Ubiquitin (Lys48-specific) (red). Actin filaments were labeled with Alexa Fluor®-488-Phalloidin (green). Nuclear material is stained with DAPI (blue).

Description	Catalogue No.
Anti-Ubiquityl-Histone H2A, clone E6C5	05-678
Ubiquitin Activating Enzyme E1	14-857
UbcH2 Conjugating Enzyme	14-807
hHR6B Conjugating Enzyme	14-854

Citrullination

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



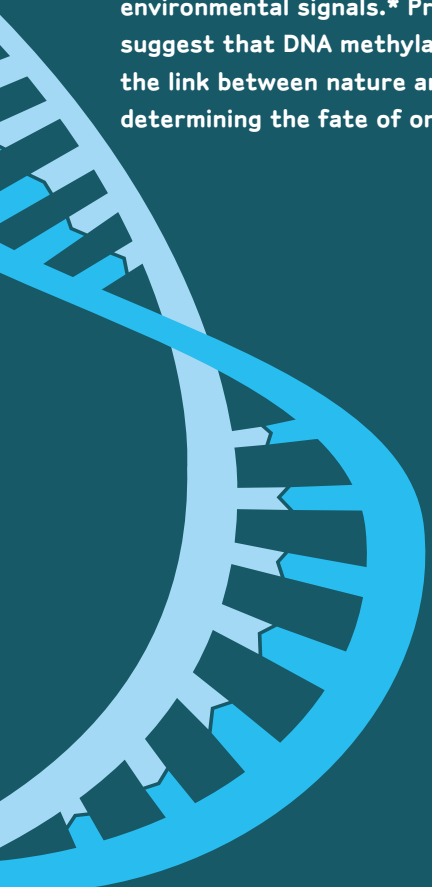
Arginine deimination regulates histone arginine methylation in HL-60 cells by converting methylarginine to citrulline. Anti-Histone H4 (citrulline 3) was used to detect citrulline in lysates of HL-60 cells treated with calcium ionophore (lane 2). Bands corresponding to citrullinated histone H4 were not observed in untreated cells (lane 1).

Description	Catalogue No.
Anti-Citrulline	07-277
Anti-Citrulline	AB5612
Anti-Citrulline (Modified) Detection Kit	17-347
Anti-Histone H4 (citrulline 3)	07-596

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. 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 is an important mechanism for gene silencing and inactivation of defined tumor suppressor genes in human cancers.

The discovery that differences between genomes cannot fully explain phenotypic differences between species or even between individuals has spurred the sequencing of "methylomes" 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.* Preliminary results suggest that DNA methylation may help complete the link between nature and nurture in determining the fate of organisms.

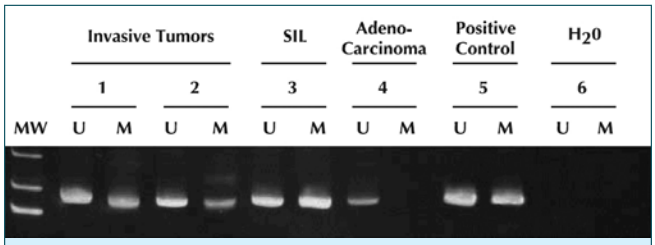


*Additional Reading:
Lister, R. and Ecker, JR. "Finding the fifth base: genome-wide sequencing of cytosine methylation." Genome Res. 2009 Jun;19(6):959-66.

CpG Islands

DNA MODIFICATION KITS FOR MAPPING METHYLATED DNA SEQUENCES

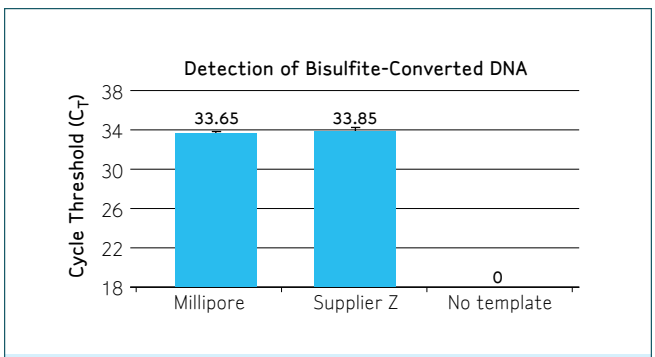
Methylation-specific PCR (MSP) is an established technique for mapping and monitoring methylation patterns in the CpG islands of genomic DNA. Millipore's CpGenome™ and CpG WIZ® systems allow sensitive detection of gene methylation using MSP of bisulfite-modified DNA. Rapid and simple, the method eliminates restriction digests and Southern blots. The CpGenome kit can detect changes in methylation patterns using as little as 1 ng of 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.

CpGenome Turbo Bisulfite Modification Kit (S7847)

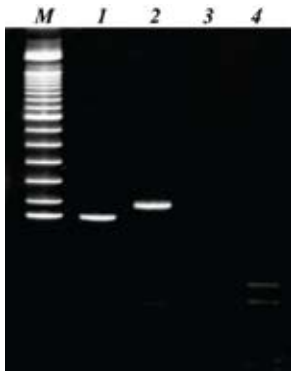
Complete bisulfite modification and DNA purification in 90 minutes. We have optimized our proprietary conversion reagent and protocol for short incubation times and >99.5% efficiency of conversion of unmethylated cytosines to uracil, starting with as little as 500 pg of DNA.



Equivalent bisulfite conversion, twice as fast. DNA (S7821) was bisulfite-converted using either the CpGenome Turbo kit or a kit from Supplier Z. 1 ng of bisulfite converted DNA was amplified using the CpG Wiz MGMT methylated primer set (S7803).

CpG WIZ MSP Kits

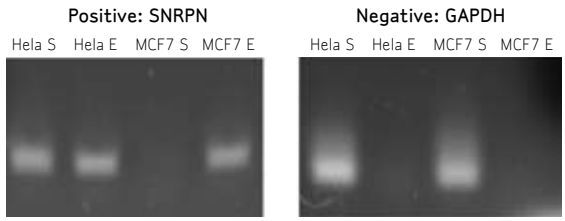
Millipore also offers over 20 CpG WIZ gene-specific, methylation-specific PCR kits, which can be accessed through www.millipore.com/epigenetics.



Specificity of the CpG WIZ Prader-Willi/Angelman amplification kit. With a complete chemical modification reaction, primers specific to unmethylated DNA amplify only unmethylated DNA (100 bp, lane 1; no bands, lane 3) and primers specific to methylated DNA amplify only methylated DNA (174 bp, lane 2; excess primer bands only, lane 4).

The CpG MethylQuest™ DNA Isolation Kit (17-10035)

Map methylation across a single gene or across the entire genome with an efficient, convenient kit for enriching methylated DNA. The CpG MethylQuest DNA isolation kit contains a GST-methyl-CpG binding domain (MBD) fusion protein coupled to magnetic beads. This unique protein tightly binds CpG methylated sequences but has extremely low affinity for non-methylated regions. With this kit you can now easily isolate methylated DNA from as little as 1 ng of DNA. Also available is unconjugated CpG MethylQuest GST-MBD, ideal for customized methylation assays.

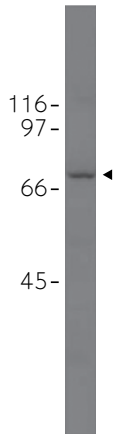


HeLa and MCF7 genomic DNA were purified with the CpG MethyQuest DNA isolation kit. 2 µL of the purified samples were used for 30 cycles of PCR amplification of SNRPN (frequently methylated locus) and GAPDH (typically unmethylated). PCR aliquots were loaded on agarose gels (S = supernatant, not bound to beads; E = elution, bound to beads)

Description	Catalogue No.
CpGenome Fast DNA Modification Kit	S7824
CpGenome Turbo Bisulfite Modification Kit	S7847
CpG WIZ Prader-Willi/Angelman Amplification Kit	S7806
CpG WIZ p16 Amplification Kit	S7800
CpG MethylMagnet DNA Isolation Kit	17-10035
CpG MethylMagnet GST-Methyl Binding Domain	14-921

DNA Methyltransferases

Millipore offers antibodies to proteins involved in DNA methylation with demonstrated performance in a variety applications. The DNA-methyltransferase enzymes (DNMT1, DNMT3a and DNMT3b) maintain normal patterns of DNA methylation. MECP2, MBD1, MBD2, MBD3, MBD4, and Kaiso each possess a MBD and act as methylation-sensitive transcriptional repressors.

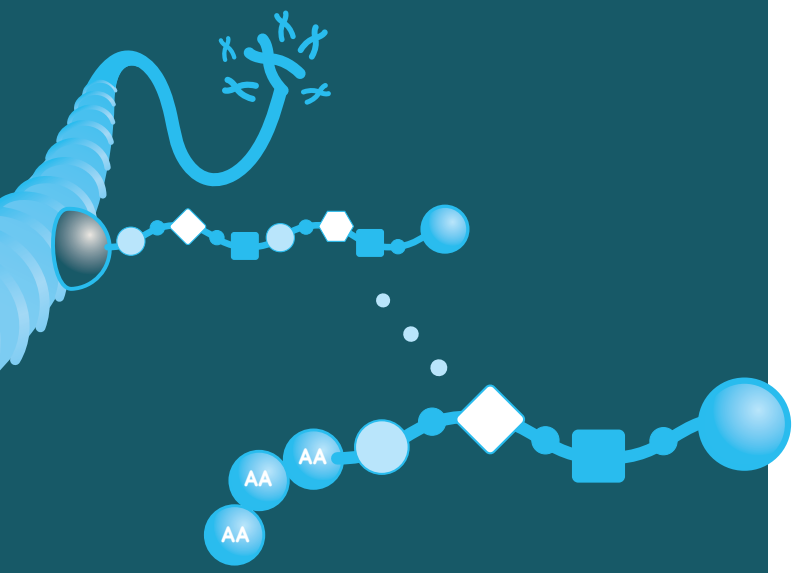


Mouse heart nuclear extract was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-MeCP2 (07-013). Proteins were visualized using a goat anti-rabbit HRP conjugate and chemiluminescence detection.

Description	Catalogue No.
Anti-DNA Methyltransferase 1	07-688
Anti-DNA Methyltransferase 2	AB3281
Anti-DNA Methyltransferase 3a	AB3431
Anti-DNA Methyltransferase 3b	AB3433
Anti-MeCP2	07-013

Transcriptional and Post-transcriptional Regulation

At the end of most cell signaling pathways lies a change in gene transcription or post-transcriptional regulation that affects the level or localization of protein expression. The proteins and RNAs that carry out these regulatory functions essentially translate the messages encoded by DNA sequence and epigenetic marks into cellular response. Characterization of the regulatory machinery, therefore, is essential for understanding and potentially modulating biological responses encoded in genomic (and epigenomic) information.



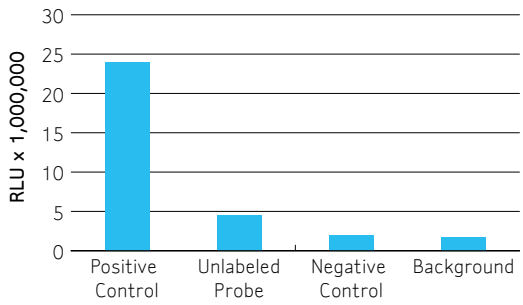
In the last decade, a new picture of gene regulatory machinery has emerged, in which transcription, RNA processing, RNA stabilization, RNA export, and even aspects of translational control are closely coupled with one another.* In the sections below, we have categorized regulatory proteins according to their most well-known function; however, each protein is likely to have multiple roles, participating in multiple stages of protein production. For example, the Hu family of RNA-binding proteins simultaneously regulates RNA stability, transport, and localization.

Transcriptional Regulation

Gene transcription is regulated by dynamic complexes of transcription factors, noncoding lincRNAs, coactivators and corepressors, histone acetylases, deacetylases, and other chromatin remodelers. Transcription factors are frequently the chief determinants of the composition and stability of these large transcription complexes, so it is important to develop robust assays to quantitate transcription factor activity.

EZ-TFA™ ASSAYS

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.



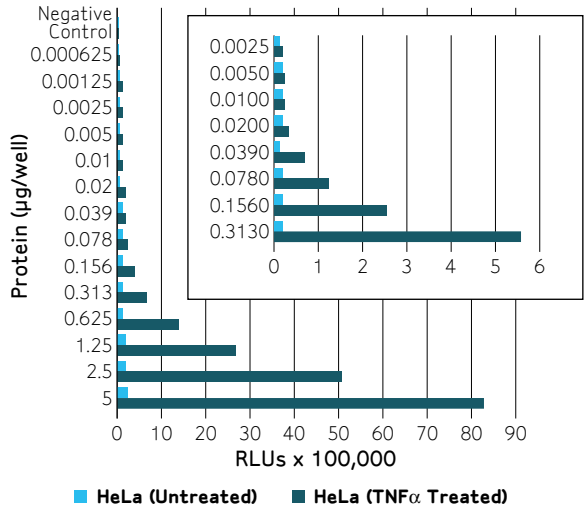
The Universal EZ-TFA kit was used to measure the DNA binding activity of the transcription factor, NFkB p65, in TNFα-stimulated HeLa cell extract. While the assay detected NFkB p65 bound to a positive control DNA probe, the binding was disrupted using unlabeled competitor probe. Minimal signal was observed using either a negative control DNA sequence or with no added DNA.

*Additional Reading

Maniatis, T. and Reed, R. "An extensive network of coupling among gene expression machines." Nature. 2002 Apr 4;416(6880):499-506.

NFκB p65 EZ-TFA Transcription Factor Assay (Chemiluminescent) (70-620)

The transcription factor NFkB (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 extract.



Demonstration of the sensitivity of the Chemiluminescent NFκB p65 Transcription Factor Assay (70-620) with lower limits of detection in the ng of nuclear protein/well quantities and the extreme dynamic range of 5 logs of magnitude of detection as demonstrated here assaying 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
Universal EZ-TFA, Chemiluminescent	70-600

Target-Specific Assays

Description	Catalogue No.
EZ-TFA NFκB p50/p65 Colorimetric/ Chemiluminescent	70-510/610
EZ-TFA AP-1 Colorimetric/Chemiluminescent	70-550/650
EZ-TFA c-Jun/c-Fos Colorimetric/ Chemiluminescent	70-546/646

Post-transcriptional Regulation

Many genes are regulated by specific post-transcriptional events. RNA-protein complexes often mediate post-transcriptional regulation, via silencing, splicing, mRNA export, and localization, as well as translation and mRNA turnover.

Recently, noncoding RNAs (ncRNAs) have been found to regulate many processes listed above. Especially during development and differentiation, ncRNAs provide a finely tuned mechanism for lineage-specific or even cell-specific protein expression. ncRNAs involved in post-transcriptional regulation include small nuclear RNAs (snRNAs), small nucleolar RNAs (snoRNAs), small interfering RNAs (siRNAs), microRNAs (miRNAs), and piwi-interacting RNAs (piRNAs).

RNA-BINDING PROTEIN IMMUNOPRECIPITATION (RIP)

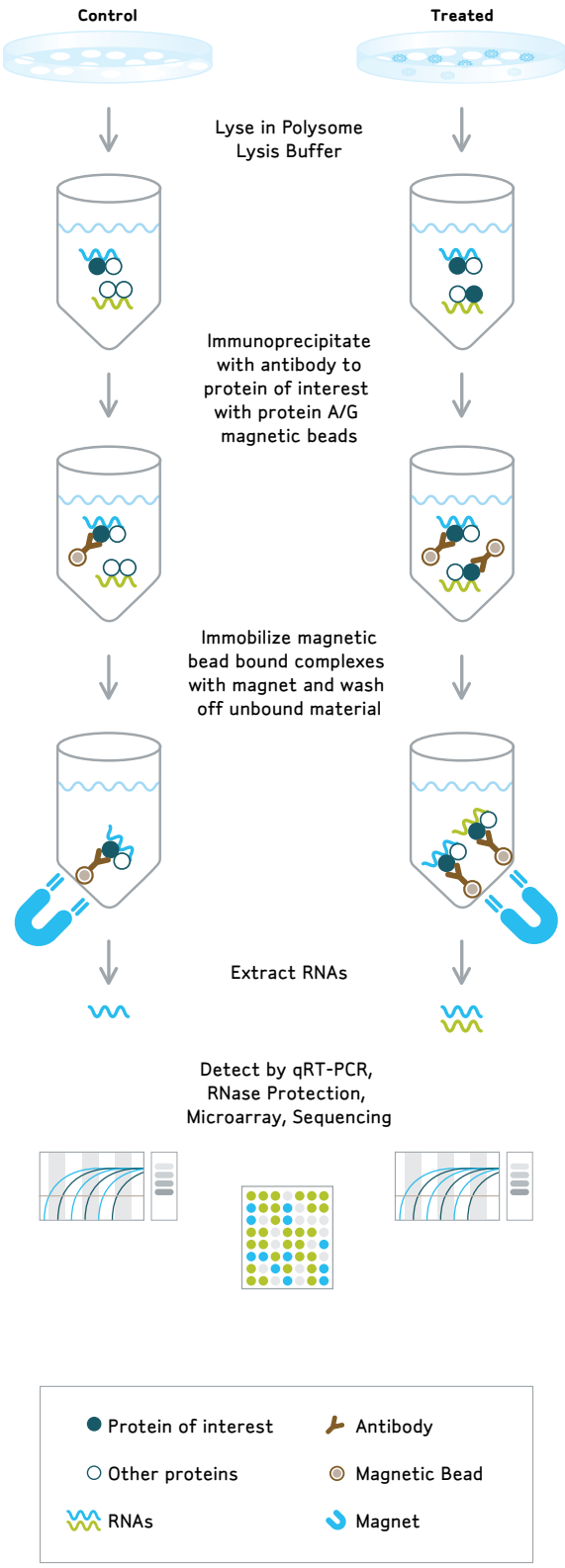
Identifying the full set of RNAs bound to particular RBPs under defined circumstances helps elucidate the role of these complexes in gene regulation. RNAs often contain more than one RBP-binding site, and each RBP can associate with multiple RNAs. Exploiting this promiscuity results in combinatorial, systems-level regulatory networks required for efficient regulation of expression. Analyzing all RNAs associated with an RBP requires genome-wide RIP followed by a global analysis of associated RNAs.

Magna RIP™ RNA-binding Protein Immunoprecipitation Kits (17-700, 17-701)

Simplify RBP immunoprecipitation (RIP) analysis using Magna RIP kits. RIP involves immunoprecipitation of RNA-binding proteins and co-isolation of RNAs within the immunoprecipitated complex (see workflow, next page). These RNA species can be interrogated and identified as mRNAs or noncoding RNAs by quantitative RT-PCR, microarray analysis (RIP-chip), or sequencing (RIP-seq). The kits are fully compatible with a wide range of RIP-validated antibodies, and contains all reagents needed for robust, specific enrichment of RBP-associated RNAs.

Description	Catalogue No.
Magna RIP RNA-binding Protein Immunoprecipitation Kit	17-700
EZ-Magna RIP Kit, including positive control antibody and primers	17-701

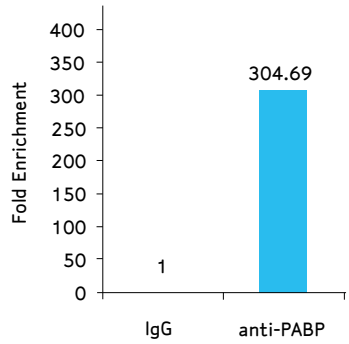
RIP Workflow



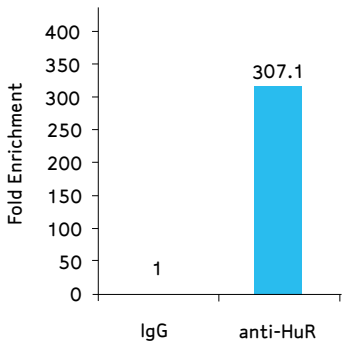
RIPAb+™ RIP-Validated Antibody Kits

Ensure the success of your RIP experiments by using RIPAb+ antibodies, which are all validated and tested for RBP immunoprecipitation. Each RIPAb+ antibody set includes a negative control antibody to guarantee specificity of the RIP reaction, and where possible, a set of control primers to biologically validate your RIP results by qPCR. Similar to our ChIPAb+ antibodies, each and every lot is validated in the RIP application.

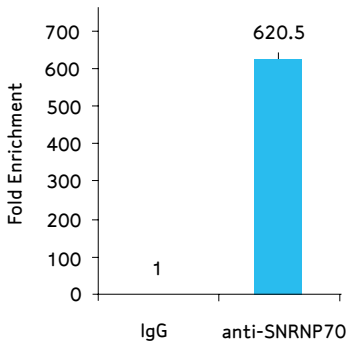
A.



B.



C.



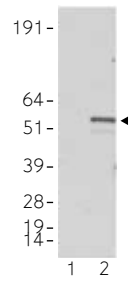
The Magna RIP Universal Kit (17-700) and three RIPAb+ antibodies and primer sets were used to study RNA binding. RIP was performed using HeLa cell lysate and anti-PABP (03-101, A), anti-HuR (03-102, B), anti-snRNP70 (03-103, C), or normal rabbit IgG as the immunoprecipitating antibody. Purified RNA was analyzed by qRT-PCR using primers specific to the actin mRNA (A, B) or U1 snRNA (C). In all three experiments, only RNA associated with the protein of interest was enriched by immunoprecipitation.

Description	Catalogue No.
RIPAb+ HuR	03-102
RIPAb+ Lin28	03-105
RIPAb+ Musashi 1*	03-114
RIPAb+ p54nrb/NonO*	03-113
RIPAb+ snRNP70	03-103

* Coming Soon

PRE-mRNA PROCESSING

After synthesis of the immature pre-mRNA strand, many other factors, including enzymes, chaperones, and noncoding RNAs, regulate the capping, trimming, and splicing of exons into the mature mRNA. These frequently overlapping processing functions not only enable the production of multiple proteins using a single gene sequence (by alternative splicing), but they also add additional checkpoints to the protein production process. As a result, a cell can coordinate a finely tuned, seemingly infinite variety of activities using a finite genome.



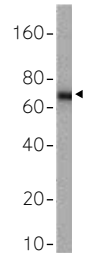
Nova-1 regulates alternative processing of neuronal pre-mRNAs. Spinal cord lysate (20 µg) from Nova-1 knockout (lane 1) and wild type (lane 2) mice were analyzed by Western blot and probed with anti-Nova-1 (07-637, 1:1000). Nova-1 is indicated by the arrow.

Antibodies for pre-mRNA Processing Factors

Description	Catalogue No.
Anti-AUF1	07-260
Anti-CUGBP1, clone 3B1	05-621
Anti-hnRNP K/J, clone 3C2	05-1519
Anti-hnRNP M1-M4, clone 1D8	05-620
Anti-hnRNP U, clone 3G6	05-1516
Anti-Nova-1	07-637

mRNA STABILITY

The abundance of an mRNA transcript in the cell is dependent on its stability, which is regulated by capping enzymes, adenylases, RNAses, and RNA-binding chaperones. Together, they prepare mRNAs for translation, and frequently work in concert with translation regulation machinery.



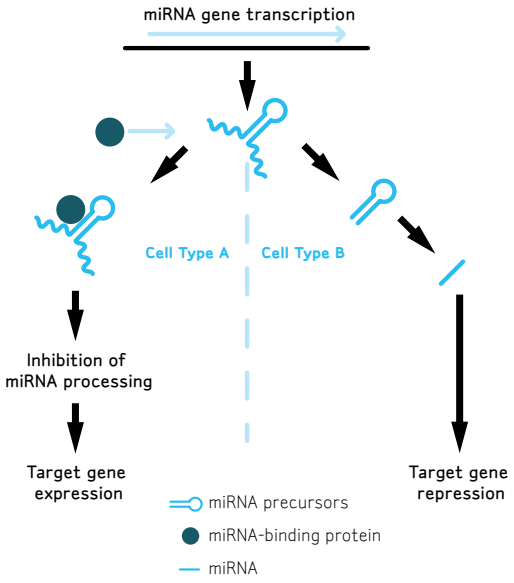
Poly-A binding protein 1 (PABP1) binds to the poly A tail of mRNA transcripts to regulate mRNA stability. Additionally, PABP1 binding is coupled to pre-mRNA processing, regulation of translation initiation, and the mRNA decay pathway. Here, PABP1 (indicated by arrow) was detected in HeLa cells by Western blotting, using the PABP1 antibody (05-847) and HRP-goat-anti-mouse Ig-G.

Antibodies for mRNA Stability Factors

Description	Catalogue No.
Anti-PABP, clone 10E10	05-847
Anti-PABPC4, clone 6E1.2	MAB11015
Anti-PUM2, clone 1E10	MAB10104
Anti-RBMS3, clone 1H6	MAB10105
Anti-RNase L, clone 2G5	05-839

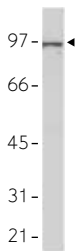
ncRNA-MEDIATED GENE REGULATION

Most commonly, ncRNAs downregulate protein expression via silencing, epigenetic changes or translational inhibition. However, ncRNAs can upregulate gene expression through certain chromatin modifications. In eukaryotic cells, miRNAs and piRNAs are the most common small RNAs that mediate gene silencing.



Role of miRNAs in cell fate determination. ncRNA-binding proteins frequently regulate ncRNA activity, and the differential expression of both ncRNAs and ncRNA binding proteins allows sophisticated patterning, for example, in developing embryos.

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. Specific knockdown of target mRNAs using designed short dsRNA sequences has become a popular genetic tool for analyzing gene function.



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.

Antibodies for ncRNA-Mediated Gene Regulation

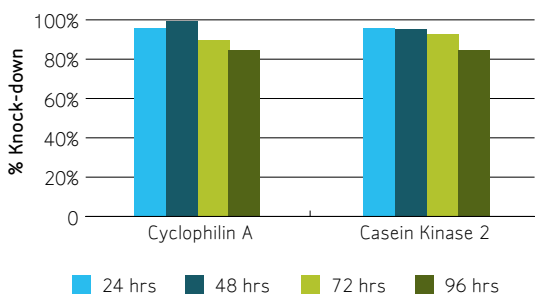
Description	Catalogue No.
Anti-Ago1	07-599
Anti-Ago1, clone 6D8.2	04-083
Anti-Ago2, clone 9E8.2	04-642
Anti-Ago4, clone 5F9.2	05-967
Anti-Dicer1, clone 5D12.2	04-721
Anti-Lin28	07-1385

RNAi TOOLS

Determining the impact of protein function on specific cellular pathways and mechanisms using gene transfer is a key technique for studying specific proteins. Since the establishment of RNAi as an effective gene silencing method, researchers have used siRNAs, either introduced into cells or transcribed from integrated DNA sequences, as molecular biology tools.

siIMPORTER™ siRNA Transfection Reagent (64-101)

Millipore's siIMPORTER siRNA transfection reagent sets a new standard for RNAi studies, cell signaling targets, providing high transfection efficiency, low cytotoxicity and guaranteed siRNA-mediated gene knockdowns. The siIMPORTER reagent works with a broad range of mammalian cells and may be used either in the presence or absence of serum in the culture medium. This versatile reagent can also be used to transfect plasmid DNA in addition to siRNAs.



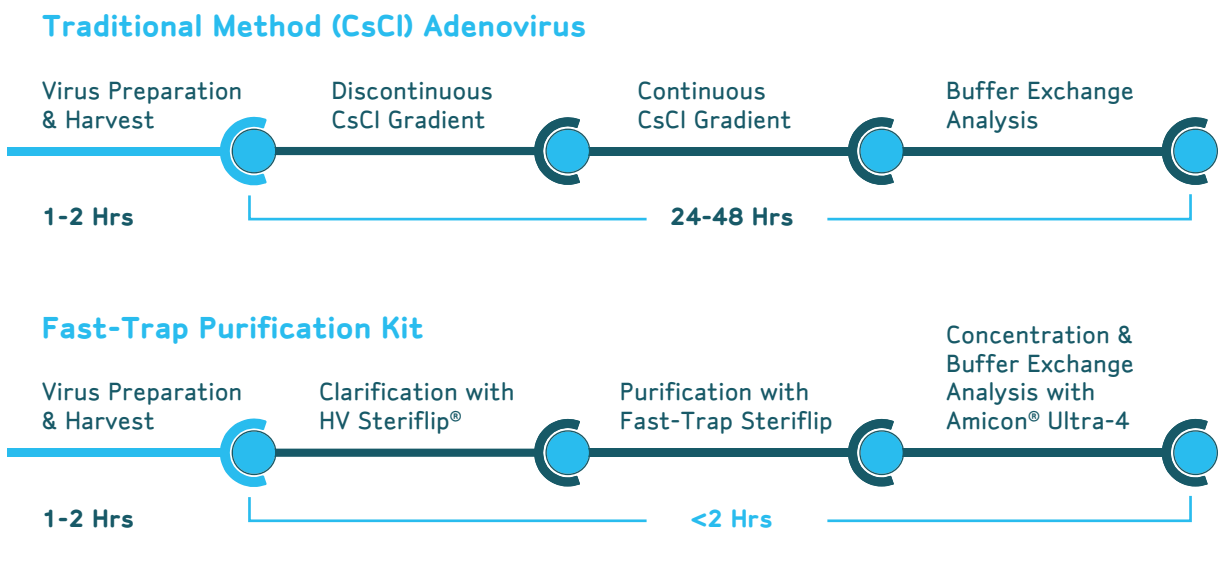
HeLa cells were transfected with siRNAs to induce silencing of cyclophilin A and casein kinase 2, using siIMPORTER siRNA Transfection Reagent (64-101). Total RNA was harvested at various time points and the transcripts of interest were measured by quantitative PCR. Results show 80-100% knockdown of both transcripts, with maximum knockdown occurring 24-48 hours after transcription.

Fast-Trap® Virus Purification Kits

For stable mRNA knockdown, short hairpin RNA (shRNA) constructs, packaged in viruses, can be transduced and integrated into cells. shRNAs can be designed to be tissue-specific, inducible, or constitutive, and transduction can be effective even for hard-to-transfect cells. However, transduction often requires virus purification, to minimize toxicity and concentrate the virus. Conventional virus purification is time-consuming, low-yielding, or both. Fast-Trap kits provide high yields of pure virus in as little as two hours, for efficient, stable mRNA knockdown.

Antibodies for ncRNA-Mediated Gene Regulation

Description	Catalogue No.
siIMPORTER siRNA Transfection Reagent	64-101
Fast-Trap Adenovirus Purification and Concentration Kit and concentrations	FTAV00003
Fast-Trap Lentivirus Purification and Concentration Kit and concentrations	FTLV00003
Fast Trap Adeno-Associated Virus (AAV) Purification and Concentration Kit	FTAA00003



Purify viruses in as little as two hours for fast, efficient transduction and stable shRNA-mediated knockdown.

mRNA TRANSPORT AND TRANSLATIONAL REGULATION

Where proteins are produced and how much protein is synthesized from each mRNA template are two of the final steps at which genes are regulated. mRNA transcripts are exported from the nucleus to various compartments of the cytosol by dynamic complexes of cellular machinery, and their entry into ribosomes is further regulated by translation initiation factors such as the eIF4 family of eukaryotic proteins.

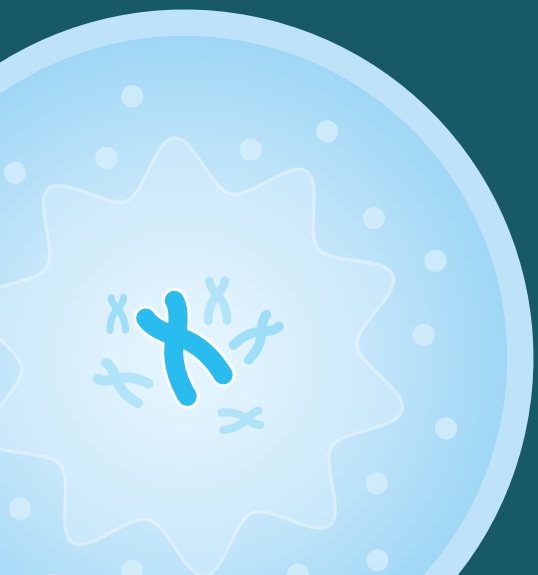
Antibodies for mRNA Transport and Translational Regulation

Description	Catalogue No.
Anti-eIF4E Binding Protein	AB3251
Anti-Fragile X Mental Retardation Protein, clone 1C3	MAB2160
Anti-MDM2, clone SMP14	MAB3776
Anti-phospho eIF4E (Ser209)	07-823
Anti-Staufen	AB5781

DNA Structure and Chromosomal Changes

DNA is organized into chromosomes, in part so that an entire genome can physically fit inside the nucleus, but also so that the cell can 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. However, 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 help elucidate the factors that cause aging, cellular degeneration, cancer, and death.



Telomere Maintenance

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 (S7700)

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.

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.

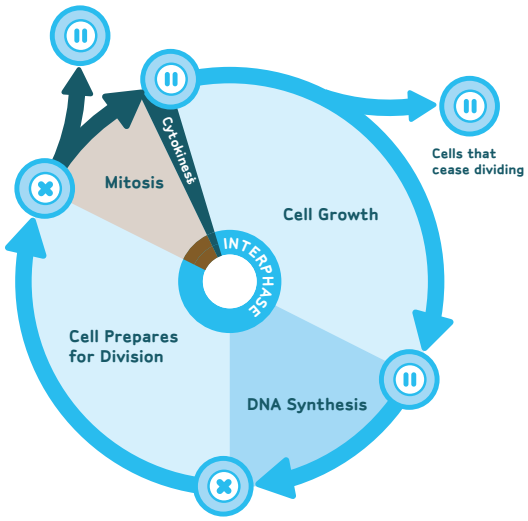
Description	Catalogue No.
TRAPeze Telomerase Detection Kit	S7700
TRAPeze XL Telomerase Detection Kit	S7707
TRAPeze ELISA Telomerase Detection Kit	S7750
TRAPeze RT Telomerase Detection Kit	S7710

Related Products

Description	Catalogue No.
TRAPeze Positive Control Cell Pellet	S7701
Anti-TRF1, clone BED5 57-6	04-638
Anti-TRF2, clone 4A794	05-521

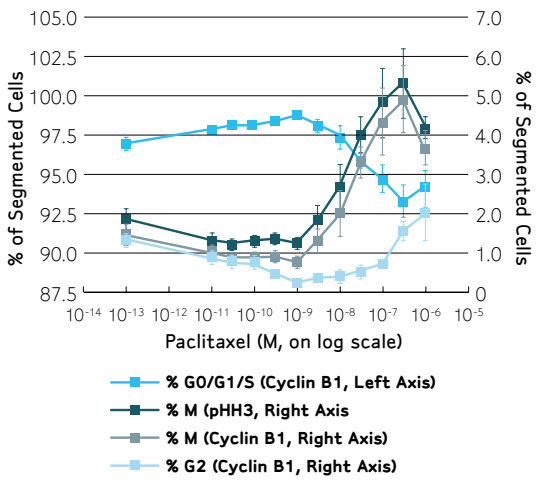
Cell Cycle

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. Millipore offers cell cycle assay kits and antibodies for the entire cell cycle research workflow.



Phospho-Histone H3 Ser10 and Cyclin B1 Assay (HCS211)

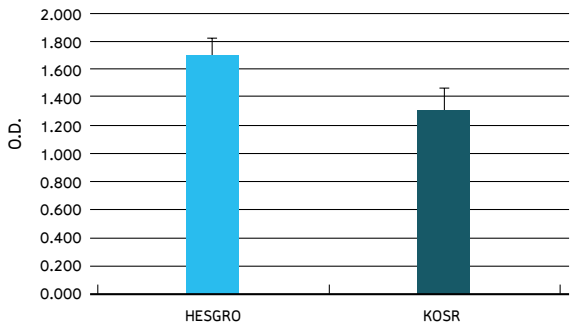
Histone H3 plays an important regulatory role in cell proliferation while cyclin B1 is a key protein in triggering mitosis. High Content Screening (HCS) of histone H3 and cyclin B1 enable distinction among G2, M, and G0/G1/S phases of the cell cycle and has been used as a readout in cell cycle inhibitor profiling studies.



Cell cycle phase analysis of HeLa cells treated for 4 hours with serial dilutions of paclitaxel. Cell handling, fixation and immunostaining were performed according to the protocol for HCS211.

BrdU Cell Proliferation Kit (2750)

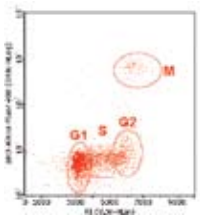
The simple, nonradioactive kit is a well-established alternative to [3H] thymidine uptake for measuring proliferation. Bromodeoxyuridine (BrdU), a photoactivatable thymidine analog, is incorporated into newly synthesized DNA strands of actively proliferating cells. After exposure to UV light, DNA strands break at sites adjacent to incorporated BrdU. These sites are then labeled with TdT and Br-dUTP, and BrdU is detected with anti-BrdU, HRP-conjugated secondary antibody, and colorimetric detection.



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

FlowCellect Bivariate Cell Cycle Kit for G2/M Flow Cytometry Analysis (FCCH025103)

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%

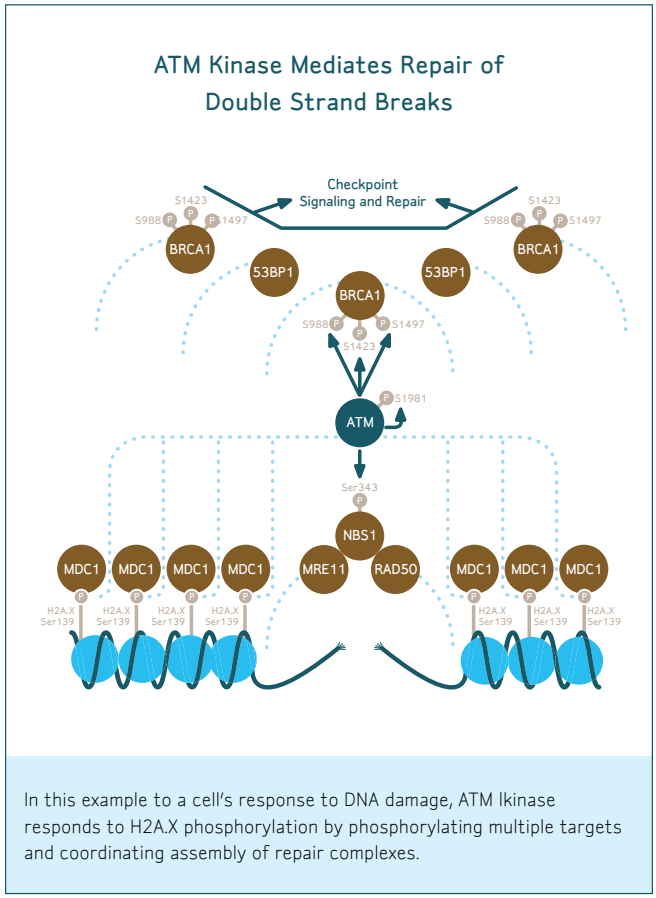
Discrimination between G2 and M phase cells by measuring the phosphorylation of Histone 3 on Ser10. Histone 3 is constitutively phosphorylated at Ser10 during metaphase.

Description	Catalogue No.
Phospho-Histone H3 (Ser10) and Cyclin B1 Assay	HSC211
BrdU Cell Proliferation Kit	2750
FlowCellect Bivariate Cell Cycle Kit for G2/M Analysis	FCCH025103

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

DNA Damage and Repair

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 reponse 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.

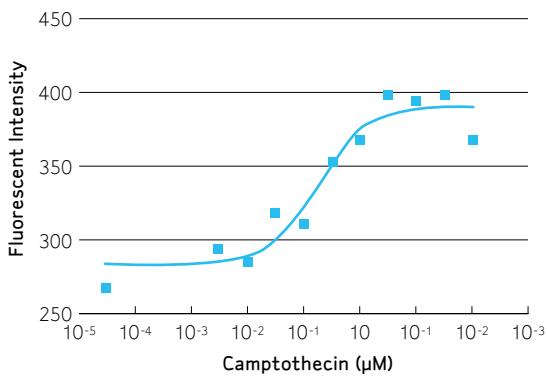


In this example to a cell's response to DNA damage, ATM Ikinase responds to H2A.X phosphorylation by phosphorylating multiple targets and coordinating assembly of repair complexes.

Multiplexed Hepatotoxicity Assay

Detecting DNA damage and repair in individual cells in response to drugs or drug candidates is an effective method for assessing drug safety or mechanism of action. However, the DNA repair pathway is seldom the only pathway activated by cytotoxic agents. Millipore's hepatotoxicity assay for human HepG2 cells is a high content screening kit for multiparametric analysis of drug-induced human cytotoxicity. In addition to visualizing the response to DNA damage by monitoring activated p53, the kit allows measurement of ten additional parameters:

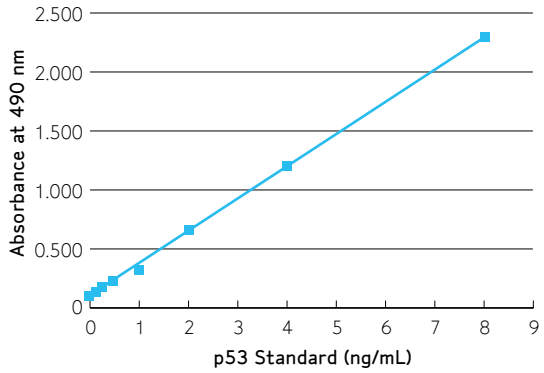
- Cell Loss
- Cell Cycle Arrest
- DNA Degradation/Apoptosis
- Nuclear Size
- Oxidative Stress
- Stress Pathway Activation
- Mitochondrial Membrane Potential
- Mitochondrial Mass
- Mitotic Arrest
- Cytoskeletal Integrity



Quantitation of high content analysis of hepatotoxicity shows increasing DNA damage response (as measured by activation of p53) with increasing concentrations of camptothecin. Data were obtained from plates imaged and analyzed using the GE IN Cell Analyzer 1000, with plots generated from GraphPad™ Prism® software.

p53 STAR ELISA Kit (17-476)

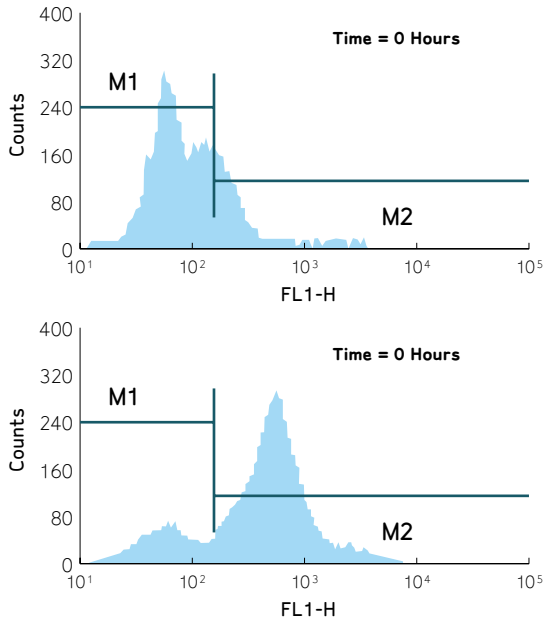
In response to DNA damage, p53 cell cycle arrest. Inactivation or loss of p53 is associated with deregulation of the cell cycle and DNA replication, inefficient DNA repair, and development of various cancers. The p53 STAR (Signal Transduction Assay Reaction) ELISA are a fast, sensitive method to detect relative amounts of total and activated p53.



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.

H2A.X Phosphorylation Assay Kit, Flow Cytometry (17-344)

Phosphorylation of the histone variant H2A.X is a rapid and sensitive response to double strand DNA breaks. This assay has been optimized for detecting levels of phosphorylated histone H2A.X via flow cytometry.



Log phase Jurkat cells were treated with 2 mg/mL staurosporine for 4 hours. Histone H2A.X (Ser139) phosphorylation was detected as described in the assay manual for the H2A.X Phosphorylation Assay Kit , Flow Cytometry (17-344) and the cells were analyzed by flow cytometry.

Description	Catalogue No.
Multiplexed Hepatotoxicity Assay	HCS100
p53 STAR ELISA Kit	17-476
H2A.X Phosphorylation Assay Kit, Flow Cytometry	17-344
Anti-Chk1	05-965
Anti-Plk1	05-844
Anti-Wee1	06-972

Additional Epigenetics Support from Millipore

With the antibody expertise of Upstate and Chemicon, Millipore offers acomprehensive portfolio of tools for studying epigenetics. Our long history and committment to innovating technologies for epigenetics is a testament to our dedication to advancing life science through steadfast customer service and high quality products. The assays featured in this brochure are only a snapshot of the entire Millipore epigenetics portfolio spanning antibodies, assays, platforms and complete solutions for research on chromatin and gene regulation. Visit our epigenetics portal at **www.millipore.com/epigenetics** fo the newest information on epigenetics and gene regulation, the latest technologies, and the most complete epigenetics product offering of any supplier.

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