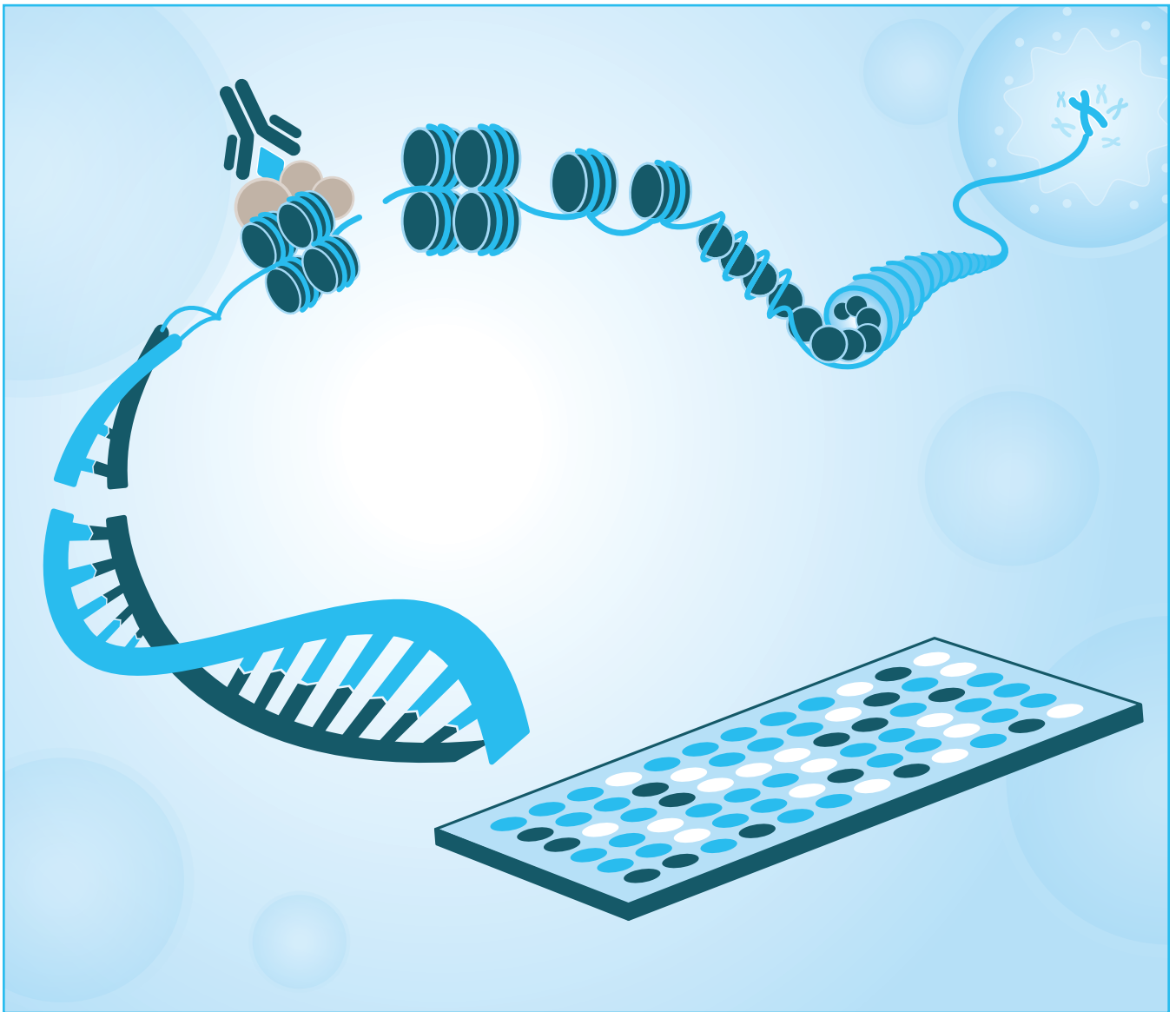




Magna ChIP²[™]

Bridge the gap between proteomics and genomics



upstate | CHEMICON

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

Introduction

Epigenetic marks are as important as DNA sequences in determining phenotypes. The study of these marks has heightened focus on protein-DNA interactions versus traditional gene-based heredity studies. Characterization of these epigenetic patterns and regulatory networks has progressed beyond standard chromatin immunoprecipitation (ChIP) -based techniques to include ChIP-chip, or genome-wide microarray analysis of ChIP-isolated DNA.

This high content approach provides insights into how regulatory and structural proteins, such as transcription factors and histone subunits, bind and interact with the genome. Immunoprecipitation of these protein-DNA complexes through high quality antibodies, (Pages 6 and 7) selective against post-translational modifications, combined with promoter or genomic tiling microarrays, identifies sequence-specific DNA binding sites with precise resolution.

ChIP-chip is a popular method for exploring chromatin structure and nuclear protein function. However, the combined techniques present challenges, both in protocol development and in reagent cost. As a result, ChIP-chip is generally limited to a small number of expertly-trained and well-funded laboratories.

Millipore's Magna ChIP² chromatin immunoprecipitation DNA microarray kits are designed to address these challenges and make ChIP-chip analysis easily accessible and affordable, with optimized reagents, user-friendly kits, and validated protocols suitable for both the novice and experienced user.

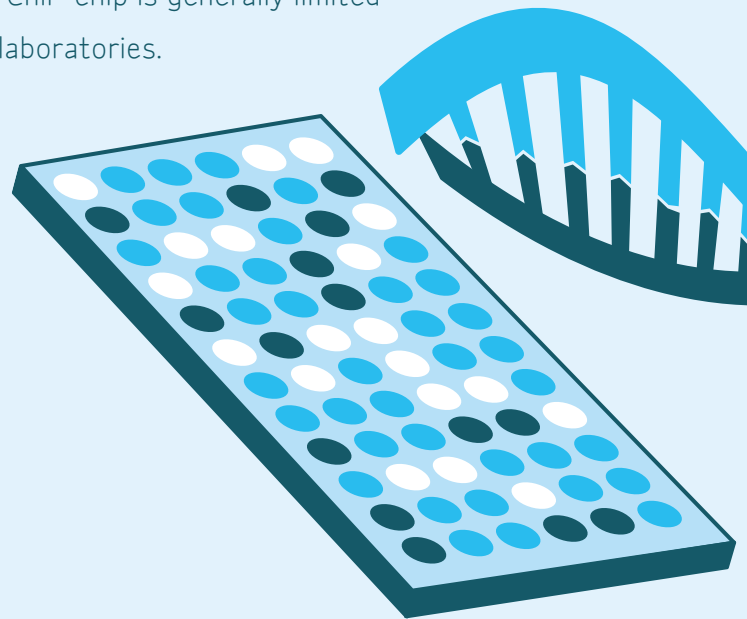
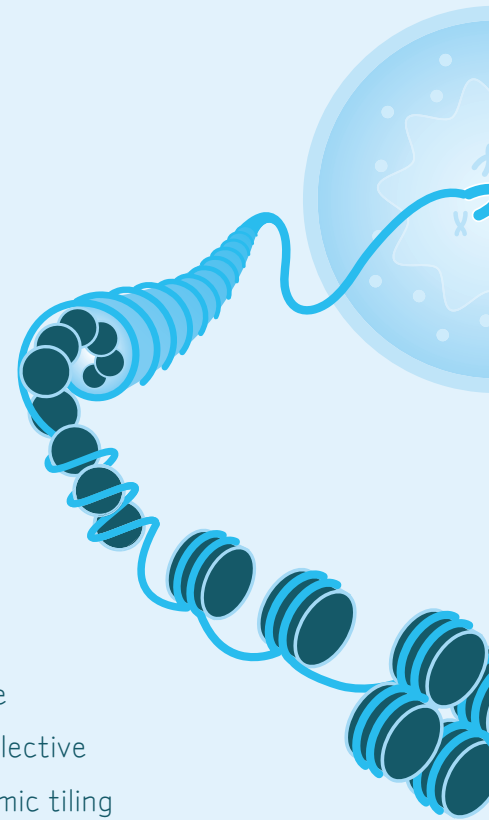


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FEATURED PRODUCTS

- Magna ChIP² Kits
- ChIPAb+ ChIP-Validated Antibody/Primer Sets
- ChIP-Qualified Antibodies



The Importance of Applying ChIP to DNA Microarrays

Transcriptional regulation is governed by a dynamic and complex interplay between proteins and genomic DNA. Histones, transcription factors, adapter proteins, polymerases, histone modifying enzymes, histone code readers as well as other proteins required for DNA maintenance and repair have established an elegant regulatory mechanisms that not only effect heritable changes in gene expression but also respond to intrinsic and external stimuli.

Determining biological function for particular protein-DNA interactions by genetics or small-scale biochemistry is difficult due to the complexity of regulatory networks, gene copy and compensation especially in higher organisms. Genome-wide analysis of these interactions or modifications provides researchers with the tools necessary to uncover the mechanisms of replication, modification, and repair and to correlate such processes with cell proliferation, cell fate, cell cycle, onco- and neurogenesis.



Applications of ChIP-chip

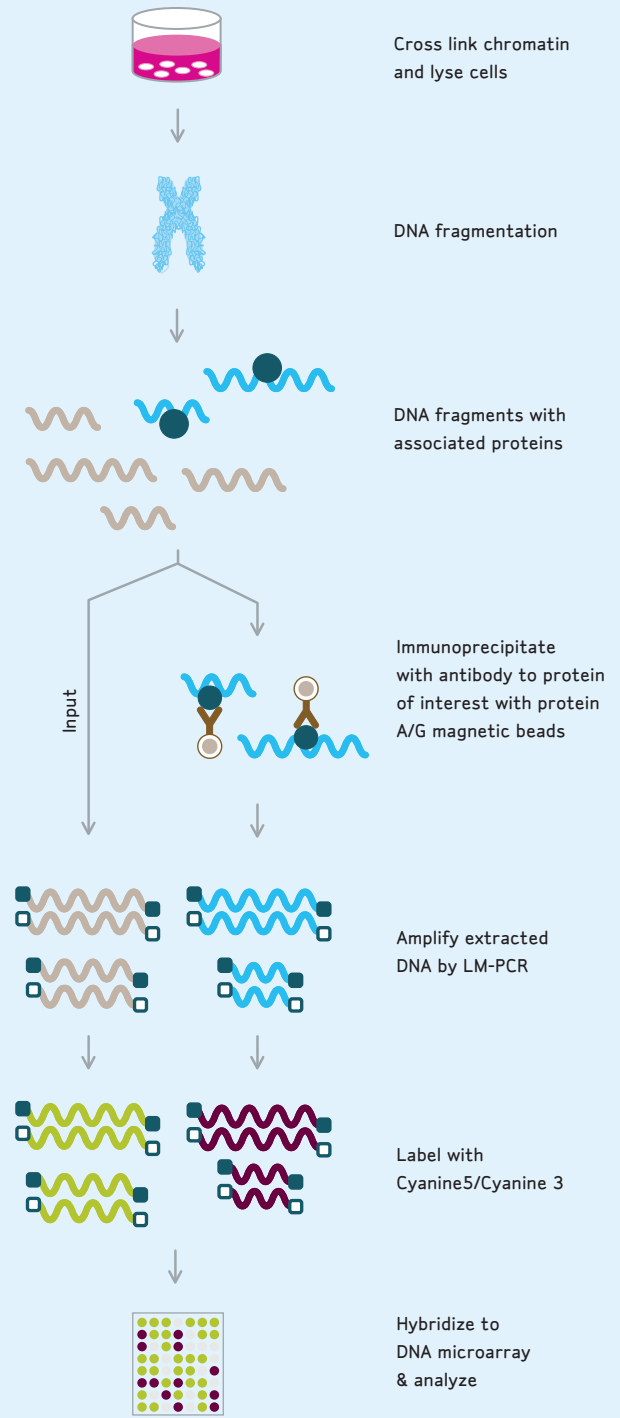
- Identify and validate networks associated with gene expression and transcriptional regulation
- Characterize macromolecular machinery influencing transcription, replication, modification and repair
- Discover changes in regulatory networks during cell development and differentiation
- Identify mechanisms of action for disease-relevant regulatory networks and screen or profile potential therapeutics

The ChIP-chip Workflow

The ChIP-chip procedure begins with immunoprecipitation of protein-DNA complexes from starting samples (ChIP), followed by amplification and hybridization of the resulting labeled pool of DNA fragments to a microarray slide for analysis.

Overview of Major Steps in ChIP-chip

1. Cross-link chromatin to capture *in vivo* snapshot of protein-DNA interactions
2. Lyse cell to release chromatin with DNA-protein complexes
3. Sonicate to fragment chromatin
4. Immunoprecipitate (IP) protein-DNA fragments using antibody against target of interest
5. Reverse DNA cross-links and amplify immunoprecipitated DNA
6. Label DNA fragments
7. Hybridize labeled DNA on to microarray slide
8. Scan microarray and perform data analysis to detect binding events



References:

1. Genome-wide location and function of DNA binding proteins. Ren B, Robert F, Wyrick JJ, Aparicio O, Jennings EG, Simon I, Zeitlinger J, Schreiber J, Hannett N, Kanin E, Volkert TL, Wilson CJ, Bell SP, Young RA. Science. 2000 Dec 22;290(5500):2306-9.
2. ChIP-chip for genome-wide analysis of protein binding in mammalian cells. Kim TH, Barrera LO, Ren B. Curr Protoc Mol Biol. 2007 Jul;Chapter 21:Unit 21.13.

Magna ChIP² Chromatin Immunoprecipitation DNA Microarray Kits

Millipore's Magna ChIP² chromatin immunoprecipitation DNA microarray 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.

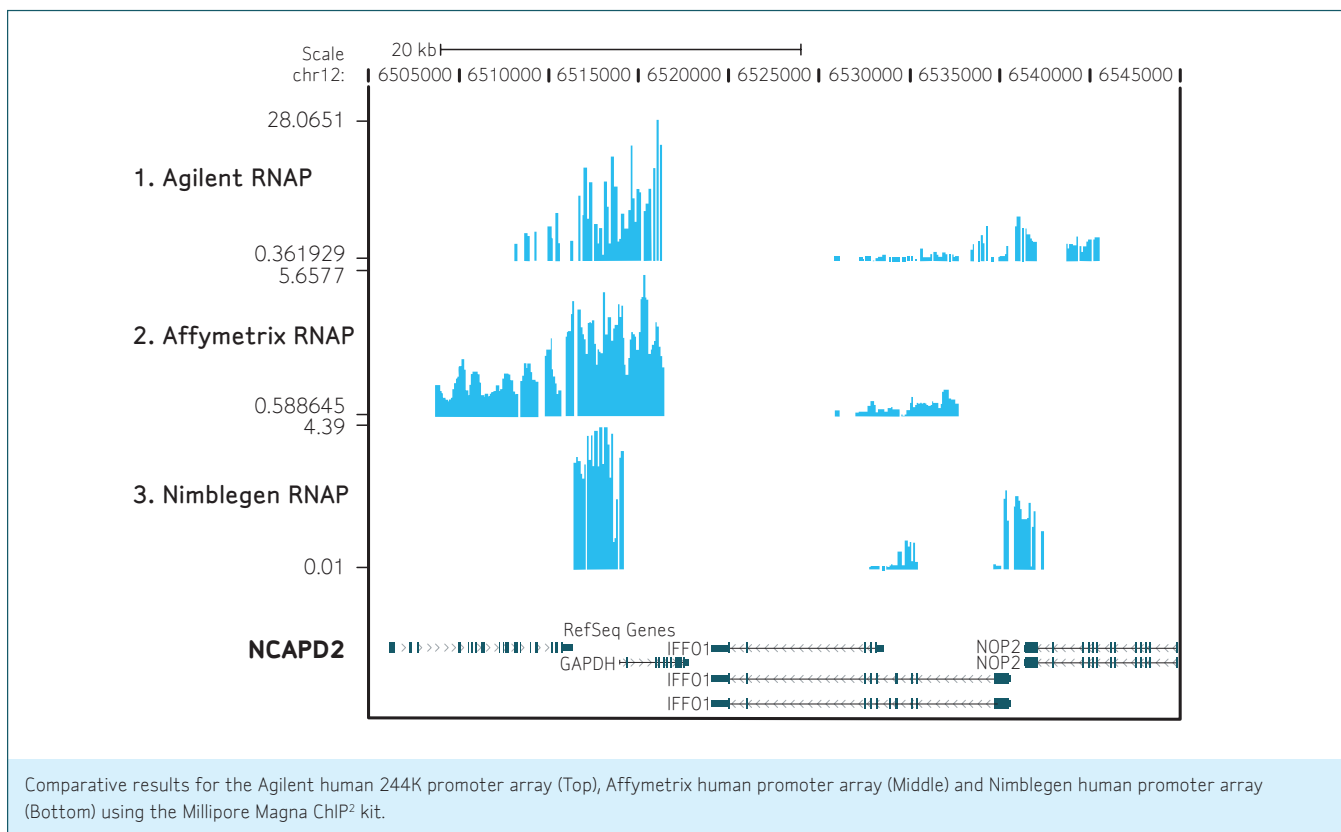
- Optimized Reagents
- Trusted & Validated Protocols
- Expert Support

PERFORMANCE

Chromatin associated with RNA polymerase II in HeLa was immunoprecipitated through use of a Millipore antibody selective for RNA polymerase II (17-620). The associated DNA was isolated and amplified using the reagents provided in the LMPCR module of the Magna ChIP² kit then labeled. The resulting fluorescent DNA probes were hybridized to the Agilent human 244K promoter array, the Affymetrix® human promoter array and the Nimblegen® human promoter array.

The resulting data was analyzed via vendor-related software and the UCSC Genome Browser (www.genome.ucsc.edu).

The Magna ChIP² kit clearly demonstrates RNA polymerase II enrichment at the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene promoter region (Figure A). Enrichment at the GAPDH promoter region is independent of the array platform; making the Millipore Magna ChIP² kit a truly universal system for ChIP-chip analysis.



ORDERING INFORMATION

Magna ChIP² Universal Kits

Optimized reagents and validated protocols for chromatin isolation and amplification, ready for labeling and hybridization to virtually any microarray.

Description	Catalogue No.
Magna ChIP ² Universal Kit (Includes materials sufficient for 6 slides)	17-1000
Magna ChIP ² Universal Quad Kit (Includes materials sufficient for 24 slides)	17-1004

Magna ChIP² Human and Mouse Promoter Kits

A complete set of reagents for ChIP-chip: isolation, amplification, labeling and hybridization, including your choice of either human or mouse Agilent promoter microarrays.

Description	Catalogue No.
Magna ChIP ² Human Promoter Kit (Includes materials sufficient for 6 slides)	17-1001
Magna ChIP ² Mouse Promoter Kit (Includes materials sufficient for 6 slides)	17-1002

ChIP-Qualified Antibodies

To complement our ChIPab+ validated antibody/primer sets, Millipore also offers a wide selection of ChIP-qualified antibodies against modified and unmodified histones, transcription factors, and other key chromatin associated proteins.

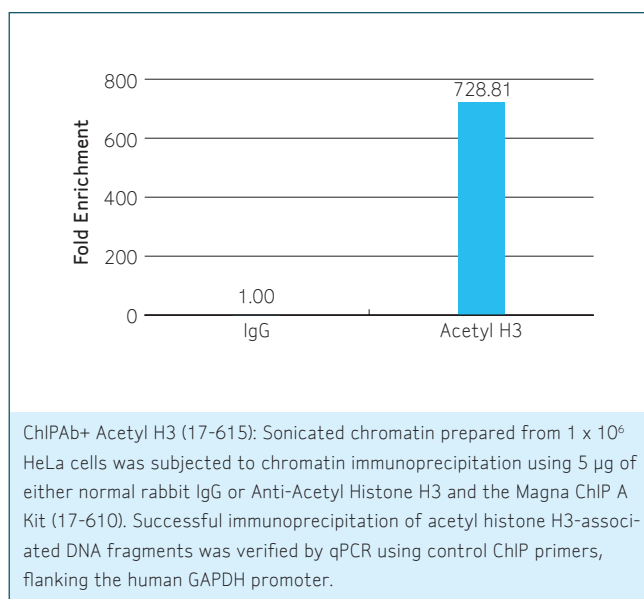
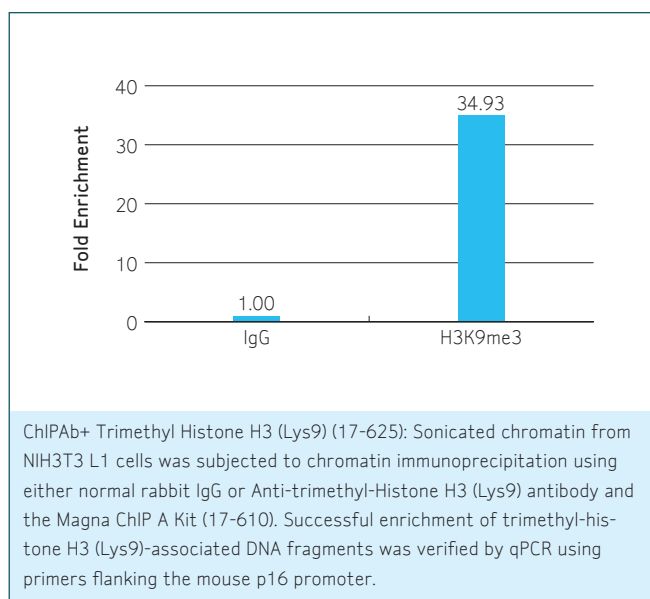
Description	Catalogue No.	Description	Catalogue No.
Anti-acetyl-Histone H2A (Lys7)	07-386	Anti-HP1 α , clone15.19s2	05-689
Anti-acetyl-Histone H2B (Lys16)	07-341	Anti-HP1 γ , clone 42s2	05-690
Anti-acetyl-Histone H3	06-599	Anti-KLF5 (Krüppel-like factor 5)	07-1580
Anti-acetyl-Histone H3 (Lys14)	07-353	Anti-LSD1	09-058
Anti-acetyl-Histone H3 (Lys18)	07-354	Anti-LSD1/BHC110	05-939
Anti-acetyl-Histone H3 (Lys23)	07-355	Anti-mono/di/trimethyl-Histone H3 (Lys4), clone AW304	05-791
Anti-acetyl-Histone H3 (Lys27)	07-360	Anti-monomethyl-Histone H3 (Lys4)	07-436
Anti-acetyl-Histone H3 (Lys9)	07-352	Anti-NF κ B p50	06-886
Anti-acetyl-Histone H4	06-598	Anti-NF κ B p65, CT	06-418
Anti-acetyl-Histone H4	06-866	Anti-ORC6, clone 3A4	05-938
Anti-acetyl-Histone H4 (Lys12)	07-595	Anti-p300 CT, clone RW128	05-257
Anti-acetyl-Histone H4 (Lys16)	07-329	Anti-PDX1	07-696
Anti-acetyl-Histone H4 (Lys5)	07-327	Anti-phospho (Ser10)-acetyl (Lys14)-Histone H3	07-081
Anti-acetyl-Histone H4 (Lys8)	07-328	Anti-phospho-Histone H2A.X (Ser139), clone JBW301	05-636
Anti-CTCF	07-729	Anti-phospho-Histone H3 (Ser10), clone MC463	04-817
Anti-dimethyl (Lys4) dimethyl (Lys9) Histone H3	07-1843	Anti-RNA polymerase II, clone CTD4H8	05-623
Anti-dimethyl-Histone H3 (Lys27)	07-421	Anti-RNA Polymerase II, CTD, clone 8WG16	05-952
Anti-dimethyl-Histone H3 (Lys36)	07-274	Anti-Sp1	07-645
Anti-dimethyl-Histone H3 (Lys36)	07-369	Anti-STAT3	06-596
Anti-dimethyl-Histone H3 (Lys4)	07-030	Anti-SUV39H1, clone MG44	05-615
Anti-dimethyl-Histone H4 (Lys20)	07-031	Anti-TATA-Binding-Protein-Associated Factor II68, a.a. 175-414	MAB3672
Anti-Histone H2A (acidic patch)	07-146	Anti-trimethyl-Histone H3 (Lys4)	07-473
Anti-Histone H2B	07-371	Anti-trimethyl-Histone H3 (Lys4), clone MC315	04-745
Anti-Histone H3	06-755	Anti-trimethyl-Histone H4 (Lys20)	07-463
Anti-Histone H3, CT, pan	07-690	Anti-ubiquityl-Histone H2A, clone E6C5	05-678
Anti-Histone H3, CT, pan, clone A3S	05-928		
Anti-Histone H4, pan, clone 62-141-13	05-858		

OUR PORTFOLIO OF CHIP-QUALIFIED ANTIBODIES IS CONSTANTLY EXPANDING!

For a complete list, visit www.millipore.com/epigenetics and search "ChIP".

ChIPAb+ ChIP Validated-Antibody/Primer Sets

All ChIPAb+ antibodies are individually validated for chromatin precipitation. Each ChIPAb+ antibody set includes control primers for locus-specific ChIP validation and a negative control antibody to confirm specificity.



Key ChIPAb+ Products

Description	Assays	Catalogue No.
ChIPAb+ Estrogen Receptor	25 assays	17-603
ChIPAb+ HDAC 1	10 assays	17-608
ChIPAb+ Lef1	25 assays	17-604
ChIPAb+ p53	25 assays	17-613
ChIPAb+ RNA Pol II	25 assays	17-620
ChIPAb+ Sp1	25 assays	17-601
ChIPAb+ Acetyl-Histone H3	25 assays	17-615
ChIPAb+ Acetyl-Histone H4	25 assays	17-630
ChIPAb+ Histone H3 (Unmodified Lys4)	25 assays	17-675
ChIPAb+ Dimethyl Histone H3 (Lys4)	25 assays	17-677
ChIPAb+ Trimethyl-Histone H3 (Lys4)	25 assays	17-614
ChIPAb+ Trimethyl-Histone H3 (Lys4)	25 assays	17-678
ChIPAb+ Acetyl-Histone H3 (Lys9) (purified)	25 assays	17-658
ChIPAb+ Monomethyl-Histone H3 (Lys9)	25 assays	17-680
ChIPAb+ Dimethyl-Histone H3 (Lys9) (serum)	25 assays	17-648
ChIPAb+ Trimethyl-Histone H3 (Lys9)	25 assays	17-625
ChIPAb+ Acetyl-Histone H3 (Lys27)	25 assays	17-683
ChIPAb+ Monomethyl-Histone H3 (Lys27)	25 assays	17-643
ChIPAb+ Trimethyl-Histone H3 (Lys27)	25 assays	17-622
ChIPAb+ Phospho-Histone H3 (Ser10), clone CMA312	25 assays	17-685

TO PLACE AN ORDER OR RECEIVE TECHNICAL ASSISTANCE

In the **U.S. and Canada**, call toll-free 1 800-Millipore (1-800-645-5476)

In **Europe**, please call Customer Service:

France: 0825.045.645

Spain: 901.516.645 Option 1

Germany: 01805.045.645

Italy: 848.845.645

English UK: 0870.900.46.45

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