Chromatin Assembly

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.

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® launched the first ChIP kits in the 1990s. Since then, we have 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, & A/G magnetic beads
- Alternate detection methods

Detection

- Quantitative PCR
- Microarray
- Sequencing
One-Day ChIP Kits

**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
- Compatible with native ChIP
- EZ-Magna ChIP™ kit with essential positive and negative control antibodies, qPCR primers

### Comparison of the One-day (Rapid) Magna ChIP™ and Standard Magna ChIP™ protocols.

<table>
<thead>
<tr>
<th>Rapid Magna ChIP™</th>
<th>Standard Magna ChIP™</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 a.m.</td>
<td>Day 1</td>
</tr>
<tr>
<td>Fix cells, harvest</td>
<td>0.5 hrs</td>
</tr>
<tr>
<td>Nuclear extraction and sonication</td>
<td>0.75 hrs</td>
</tr>
<tr>
<td>Immunoprecipitation</td>
<td>Overnight</td>
</tr>
<tr>
<td>IP wash, elution, crosslink reversal</td>
<td>3.0 hrs</td>
</tr>
<tr>
<td>DNA cleanup and PCR</td>
<td>3.0 hrs</td>
</tr>
<tr>
<td>6 p.m.</td>
<td>Day 2</td>
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</table>

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.

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 10⁶ 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 IkBo.

### Magnetic Bead-based Kits
- Magna ChIP™ A/G Kit 17-10085
- EZ-Magna ChIP™ A/G Kit 17-10086
- Magna ChIP™ HiSens Chromatin Immunoprecipitation Kit 17-10460
- EZ-Magna ChIP™ A 17-408
- EZ-Magna ChIP™ G 17-409

### Agarose Bead-based Kits
- ChIP Assay Kit 17-295
- Acetyl-Histone H3 Immunoprecipitation (ChIP) Assay Kit 17-245
- Acetyl-Histone H4 Immunoprecipitation (ChIP) Assay Kit 17-229

### GenElute™ Binding Column G
- Imprint® Chromatin Immunoprecipitation Kit CHPI
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

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**Minimal Well-to-well Carryover Contamination**

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.

<table>
<thead>
<tr>
<th>Description</th>
<th>Cat. No.</th>
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<tbody>
<tr>
<td>Magna ChIP™ HT96</td>
<td>17-10077</td>
</tr>
<tr>
<td>Magna ChIP™ HT96 ChIP Plate Set</td>
<td>17-10459</td>
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</table>

**ChIP-Validated Antibodies and Primer Sets**

**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) (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

<table>
<thead>
<tr>
<th>Description</th>
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<tr>
<td>ChIPAb+™ Histone H2A.Z</td>
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<td>ChIPAb+™ Histone H2B</td>
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<td>ChIPAb+™ Histone H3 (C-term)</td>
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<tr>
<td>ChIPAb+™ Histone H3 (Unmod Lys4)</td>
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<td>ChIPAb+™ Acetyl Histone H3</td>
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<tr>
<td>ChIPAb+™ Acetyl-Histone H3 (Lys4)</td>
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<td>ChIPAb+™ Acetyl-Histone H3 (Lys9)</td>
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<tr>
<td>ChIPAb+™ Acetyl-Histone H4 (Lys5)</td>
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<td>ChIPAb+™ CTCF</td>
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<td>ChIPAb+™ EED</td>
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<tr>
<td>ChIPAb+™ EED (Rabbit Polyclonal)</td>
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<td>ChIPAb+™ ERa</td>
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<tr>
<td>ChIPAb+™ EZH2, clone AC22</td>
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<tr>
<td>ChIPAb+™ HDAC1</td>
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<tr>
<td>ChIPAb+™ p53</td>
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<tr>
<td>ChIPAb+™ Phospho-CREB (Ser133)</td>
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<td>ChIPAb+™ REST</td>
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</table>

### Accessories

#### 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.

#### 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

#### 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.
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 nuclear RIP™ ImmunoPrecipitation Kits

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

<table>
<thead>
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<tr>
<td>Magna RIP™ Kit, 12 reactions</td>
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<tr>
<td>EZ-Magna RIP™ RNA-Binding Protein Immunoprecipitation Kit</td>
<td>17-701</td>
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</tbody>
</table>

Magna RIP™ and EZ-Magna nuclear RIP™ ImmunoPrecipitation Kits

- Protein A/G magnetic bead blend
- Compatible with an extensive line of RIPAb+™ validated antibodies
- A complete set of optimized reagents including RNase inhibitors
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<td>Magna RIP™ Quad RNA-Binding Protein Immunoprecipitation Kit</td>
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<td>Magna Nuclear RIP™ (Cross-Linked) Nuclear RNA-Binding Protein Immunoprecipitation Kit</td>
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<td>EZ-Magna nuclear RIP™</td>
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<tr>
<td>Magna Nuclear RIP™ (Native) Nuclear RNA-Binding Protein Immunoprecipitation Kit</td>
<td>17-10522</td>
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<tr>
<td>EZ-Magna Nuclear RIP™ (Native) Nuclear RNA-Binding Protein Immunoprecipitation Kit</td>
<td>17-10523</td>
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<tr>
<td>Magna MeRIP™ m6A Kit- Transcriptome-wide Profiling of N6-Methyladenosine</td>
<td>17-10499</td>
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</tbody>
</table>
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.

![Confocal IF analysis of HeLa, NIH 3T3 using anti-HuR (Red). Actin filaments have been labeled with AlexaFluor® 488 -Phalloidin (Green). Nucleus is stained with DAPI (Blue).](image1)

![Confocal IF analysis of HeLa, NIH 3T3 using anti-HuR (Red). Actin filaments have been labeled with AlexaFluor® 488 -Phalloidin (Green). Nucleus is stained with DAPI (Blue).](image2)

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### Description | Cat. 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+™ 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

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).