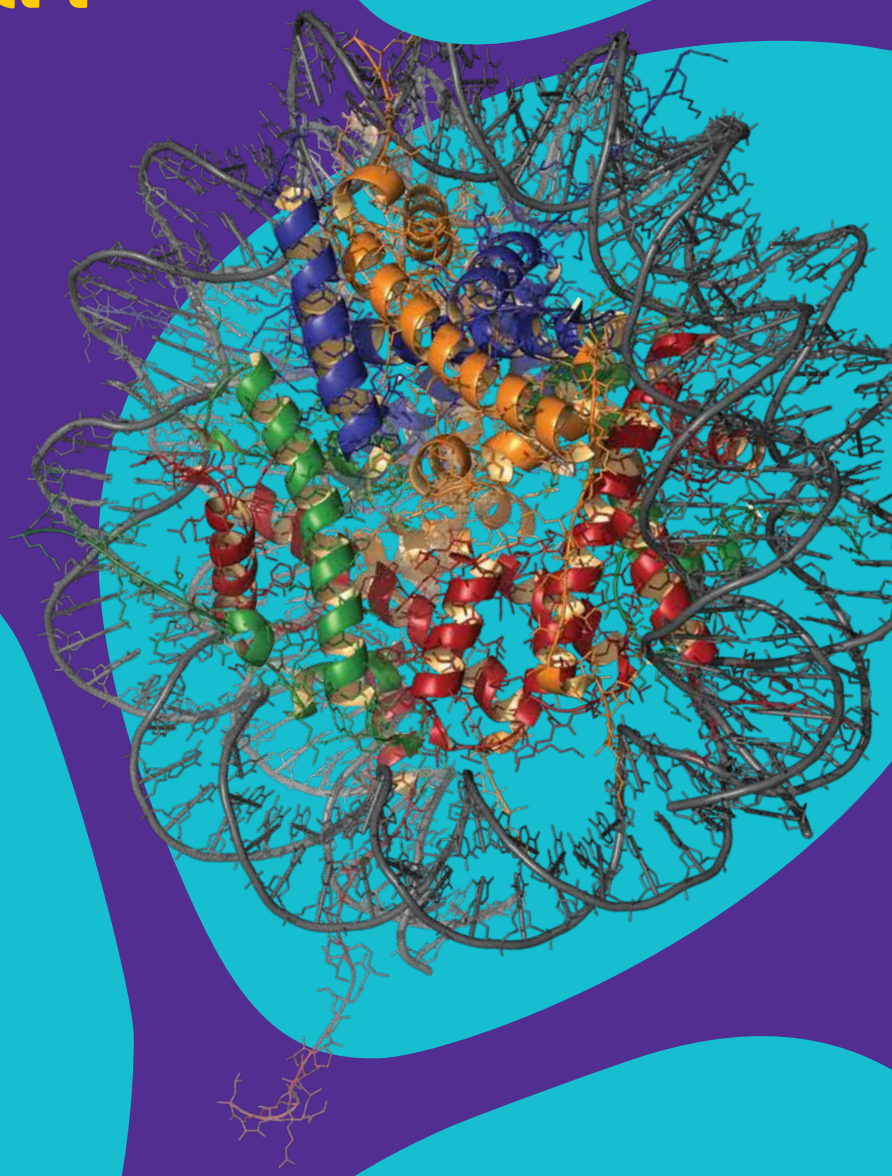
MilliporeSigma

chromatin analyses

Product Selection Guide



The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

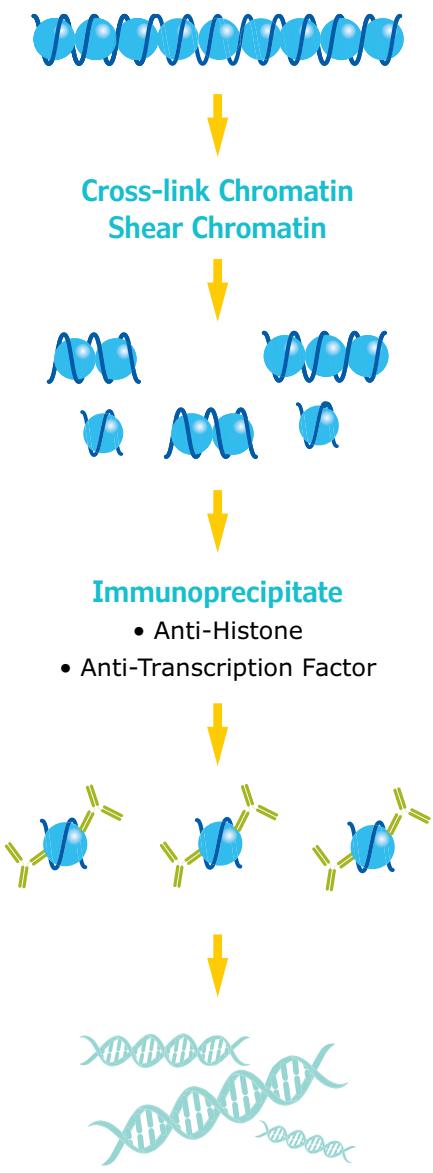
Chromatin Assembly

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.

Chromatin IP Technique



A HISTORY OF INNOVATION

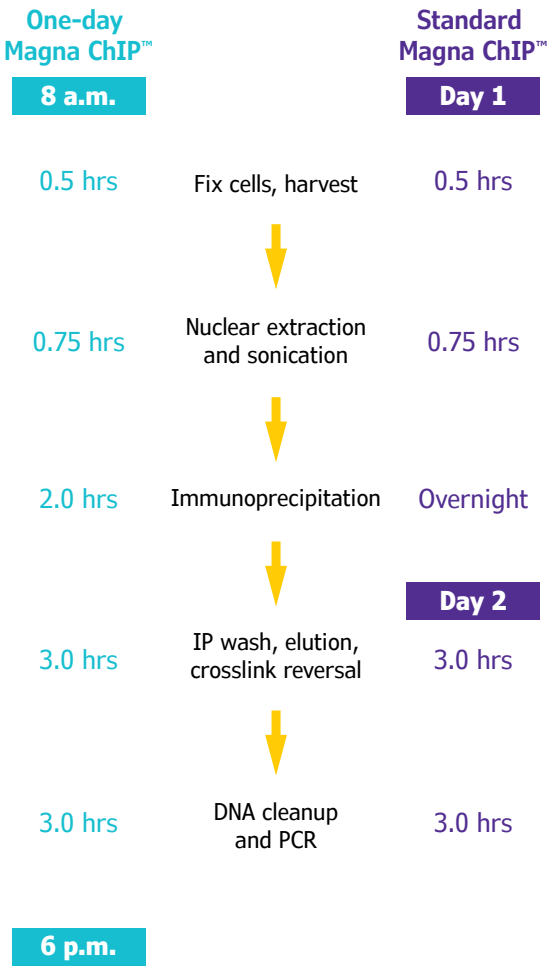
Upstate® launched the first ChIP kits in the 1990s. Since then, we have introduced an extensive line of ChIP technologies with many advantages:

- 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

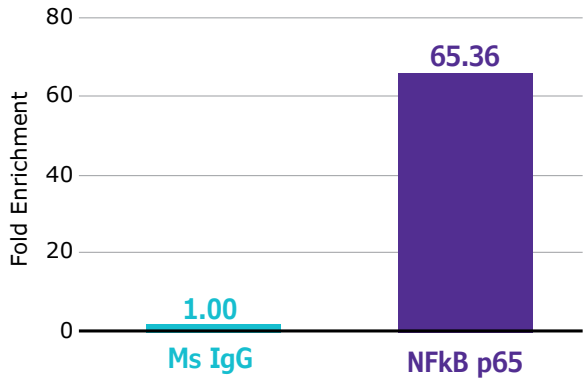
One-Day ChIP Kits

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. 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 IκBα.

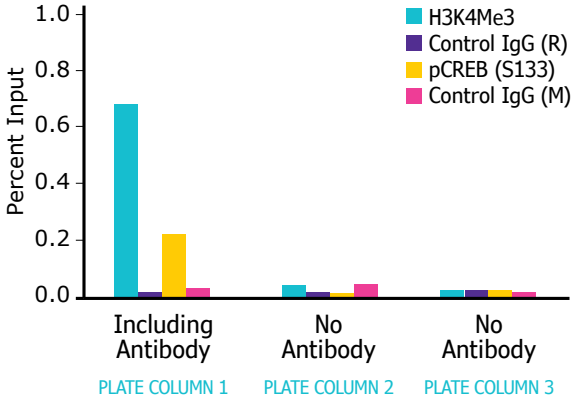
Description	Cat. No.
Magnetic Bead-based Kits	
Magna ChIP™ A/G Kit	17-10085
EZ-Magna ChIP™ A/G Kit	17-10086
Magna ChIP™ HiSens Chromatin Immunoprecipitation Kit	17-10460
EZ-Magna ChIP™ A	17-408
EZ-Magna ChIP™ G	17-409
Agarose Bead-based Kits	
ChIP Assay Kit	17-295
Acetyl-Histone H3 Immunoprecipitation (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

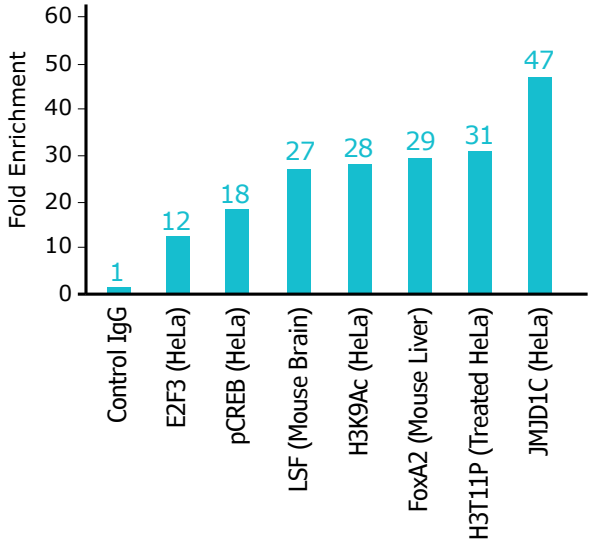
- Up to 96 ChIP reactions at once
- ChIP using cells or tissue
- Multichannel pipette or automated protocols
- Protein A/G magnetic bead blend
- EZ-Magna ChIP™ kit with essential positive and negative control antibodies, qPCR primers
- Efficient and reproducible
- Technically demanding ChIP made easy

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.

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.

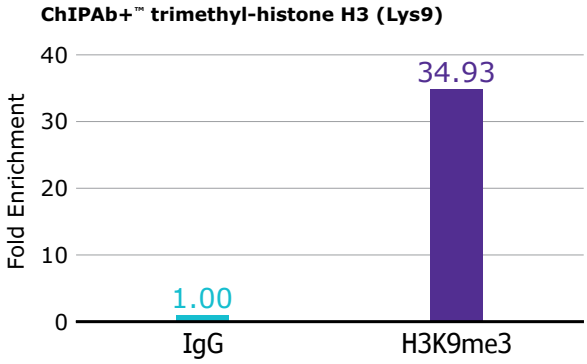
Description	Cat. No.
Magna ChIP™ HT96	17-10077
Magna ChIP™ HT96 ChIP Plate Set	17-10459

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	Cat. 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

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

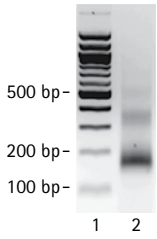
Choose one of our magnetic racks for Magna ChIP™ assays: the classic Magna GrIP™ rack, the extra-strong, contoured PureProteome™ magnetic stands, or the new Magna GrIP™ HT96 rack, which is ideal for high throughput ChIP.

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.

Description	Cat. No.
EZ-Zyme™ Chromatin Preparation kit	17-375
Magna ChIP™ Protein A+G Magnetic Beads	16-663
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

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

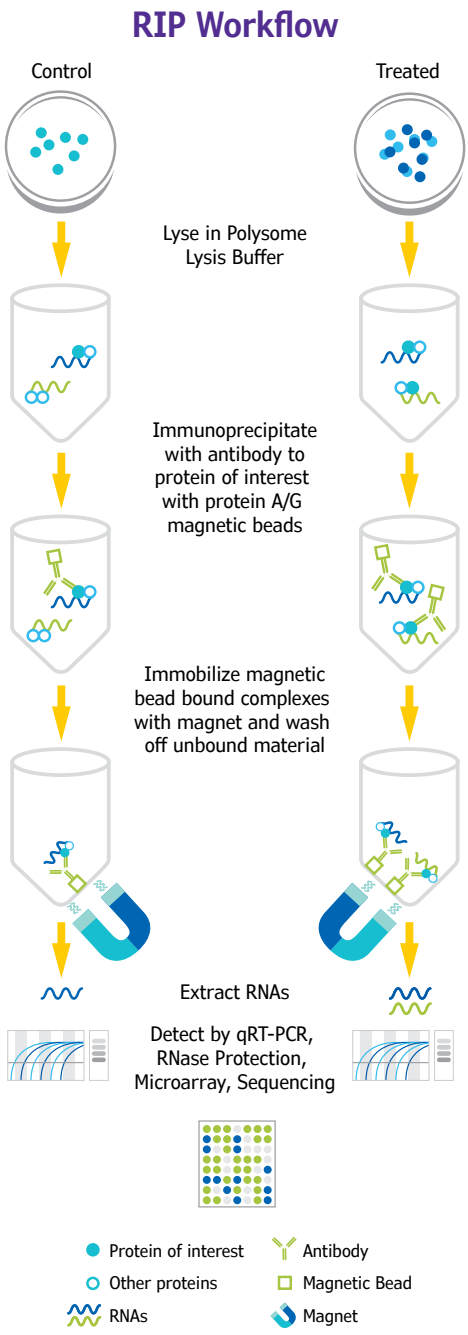
RNA-binding Protein Immunoprecipitation (RIP)

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

Description	Cat. No.
Magna RIP™ Kit, 12 reactions	17-700
EZ-Magna RIP™ RNA-Binding Protein Immunoprecipitation Kit	17-701



Description	Cat. No.
Magna RIP™ Quad RNA-Binding Protein Immunoprecipitation Kit	17-704
Magna Nuclear RIP™ (Cross-Linked) Nuclear RNA-Binding Protein Immunoprecipitation Kit	17-10520
EZ-Magna nuclear RIP™	17-10521
Magna Nuclear RIP™ (Native) Nuclear RNA-Binding Protein Immunoprecipitation Kit	17-10522
EZ-Magna Nuclear RIP™ (Native) Nuclear RNA-Binding Protein Immunoprecipitation Kit	17-10523
Magna MeRIP™ m6A Kit- Transcriptome-wide Profiling of N6-Methyladenosine	17-10499

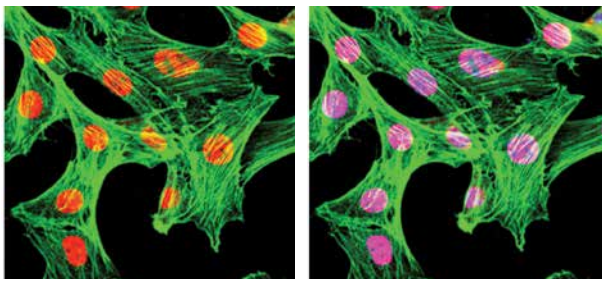
RIPAb+™ Antibody/Primer Sets

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.

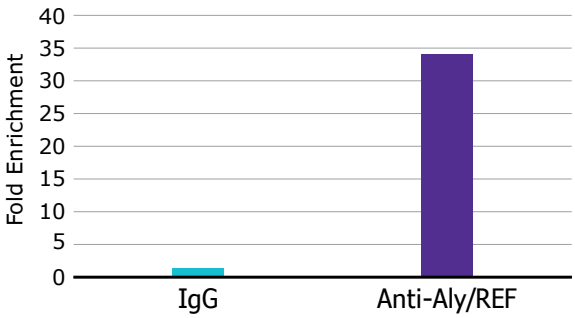
RIPAb+™ HuR

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 filaments 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	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

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