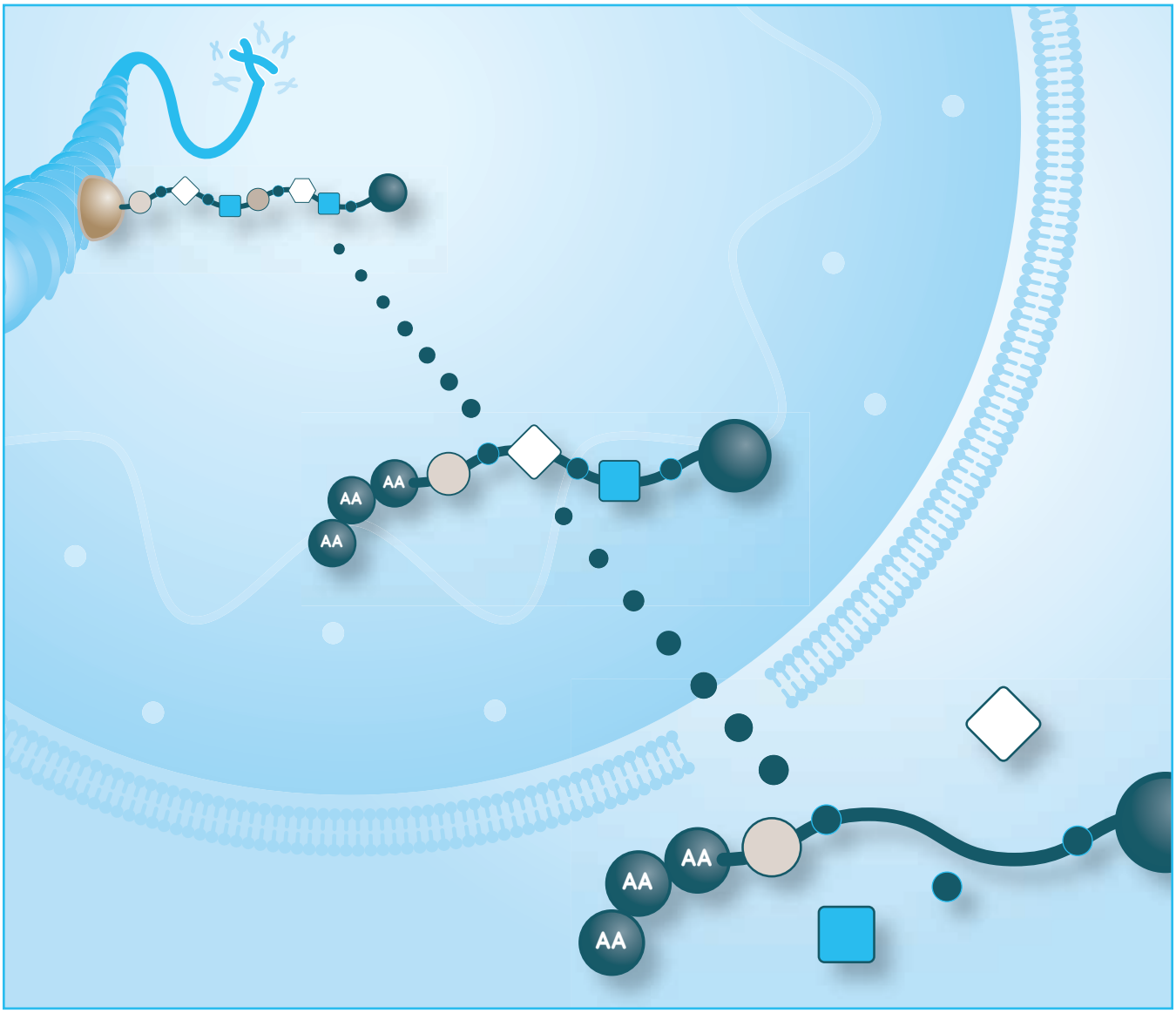
MILLIPORE™

RNA-Binding Protein Immunoprecipitation

Validated Kits, Antibodies, and Accessories



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Introduction

All eukaryotic organisms require tight regulation of gene expression for complex processes such as development, differentiation, cell specification, and responses to environmental stimuli. Many genes are regulated post-transcriptionally, in addition to transcriptional mechanisms of gene regulation. RNA-binding proteins (RBPs) are essential for post-transcriptional gene regulation, linking transcription and translation in many processes including transcription, splicing, export, rate of translation and turnover. In all of these events, RBPs coordinate regulation of the amount of protein produced from mRNA transcripts.

The number of identified RBPs has surged in recent years. Their biological importance is clear, and much work now focuses on how they act, interact with one another, and are controlled. An important next step will be identifying the full complement of RNAs associated with particular RBPs under defined circumstances.

Guiding the direction of translational regulation research are two attributes of RNA-protein complexes. First, RNAs often contain binding sites for more than one RBP; second, each RBP can associate with multiple RNAs. Exploiting these two attributes results in combinatorial, systems-level regulatory networks with the potential for building complex yet elegant cellular circuits.

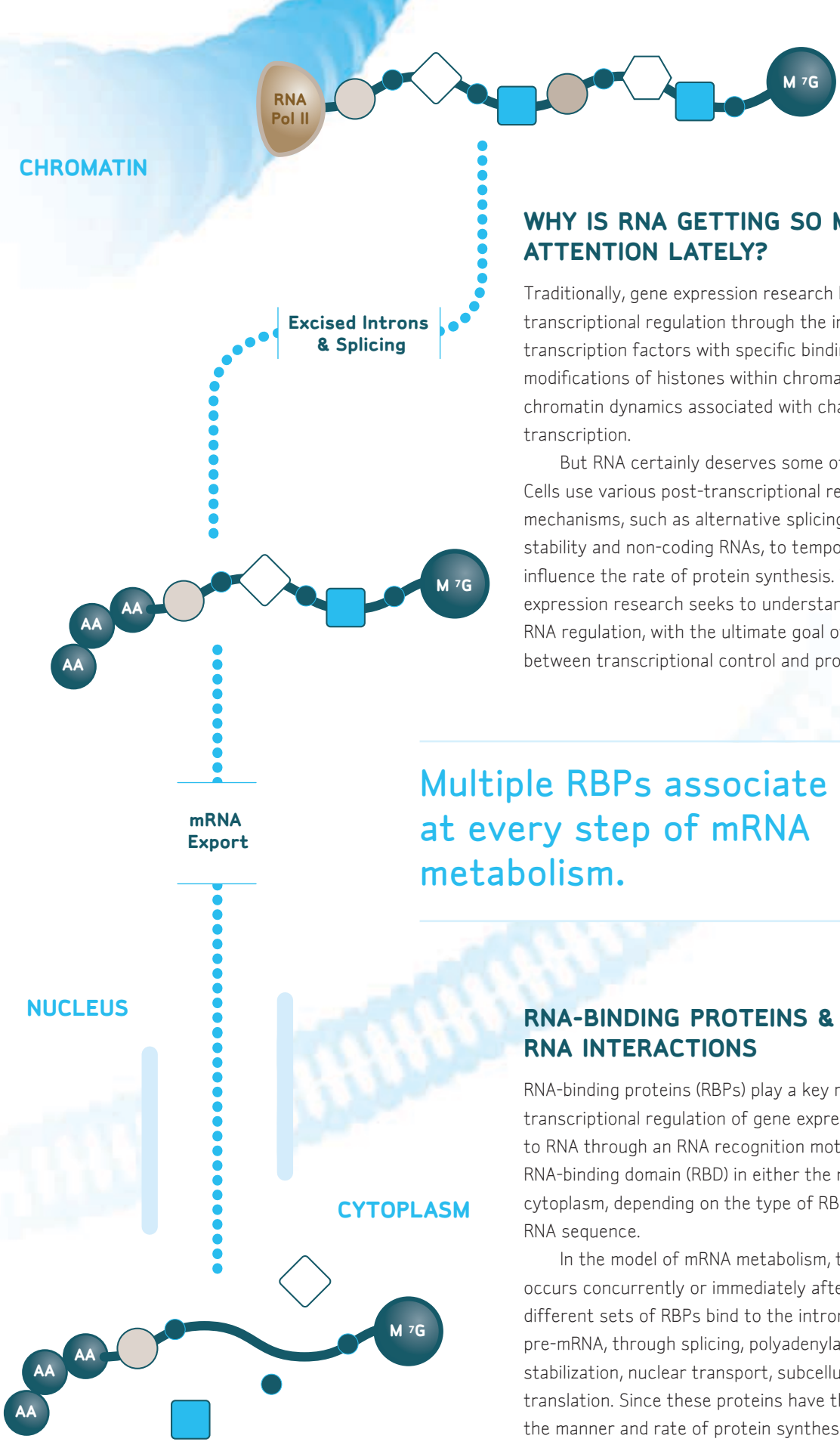
Analyzing the entire subset of RNAs associated with a particular RBP requires the optimization of new RNA-binding protein immunoprecipitation (RIP) methods. This emerging technique, which employs immunoprecipitation of ribonucleoprotein (RNP) complexes using an antibody specific to the RBP of interest, can be paired with a genome-based, global analysis of associated RNAs. Since RIP can also detect RNAs that do not directly bind to a particular RBP but may form part of the larger RNP complex, the technique can help reveal the RNA infrastructure of a cell or tissue. A growing body of literature documents the use of RIP to profile RNAs associated with selected RBPs, thus supporting it as a fundamental technique for dissecting post-transcriptional gene regulation.

The identification of RNA-RBP networks and RNA sites (genic as well as non-genic) bound by RBPs is essential for fully understanding the complex regulatory processes that govern gene expression, and will provide credence to the observation that RBPs are important for cellular regulation.

TABLE OF CONTENTS

FEATURED PRODUCTS

- Magna RIP Kits
- RIPAb+™ Validated Antibody/Primer Sets
- RBP Antibodies
- Biopak™ Point-of-use Ultrafilter



WHY IS RNA GETTING SO MUCH ATTENTION LATELY?

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.

But RNA certainly deserves some of the spotlight too. 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.

Multiple RBPs associate at every step of mRNA metabolism.

RNA-BINDING PROTEINS & RNA INTERACTIONS

RNA-binding proteins (RBPs) play a key role in post-transcriptional 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.

In the model of mRNA metabolism, this interaction occurs concurrently or immediately after transcription as different sets of RBPs bind to the introns and exons of pre-mRNA, through splicing, polyadenylation, mRNA stabilization, nuclear transport, subcellular localization and translation. Since these proteins have the potential to affect the manner and rate of protein synthesis, it is crucial to have a reliable method for identifying and characterizing these RBP/RNA interactions. (For a list of RBP antibodies available from Millipore, see page 7).

ANALYZING PROTEIN-RNA INTERACTIONS BY RNA-BINDING PROTEIN IMMUNOPRECIPITATION (RIP)

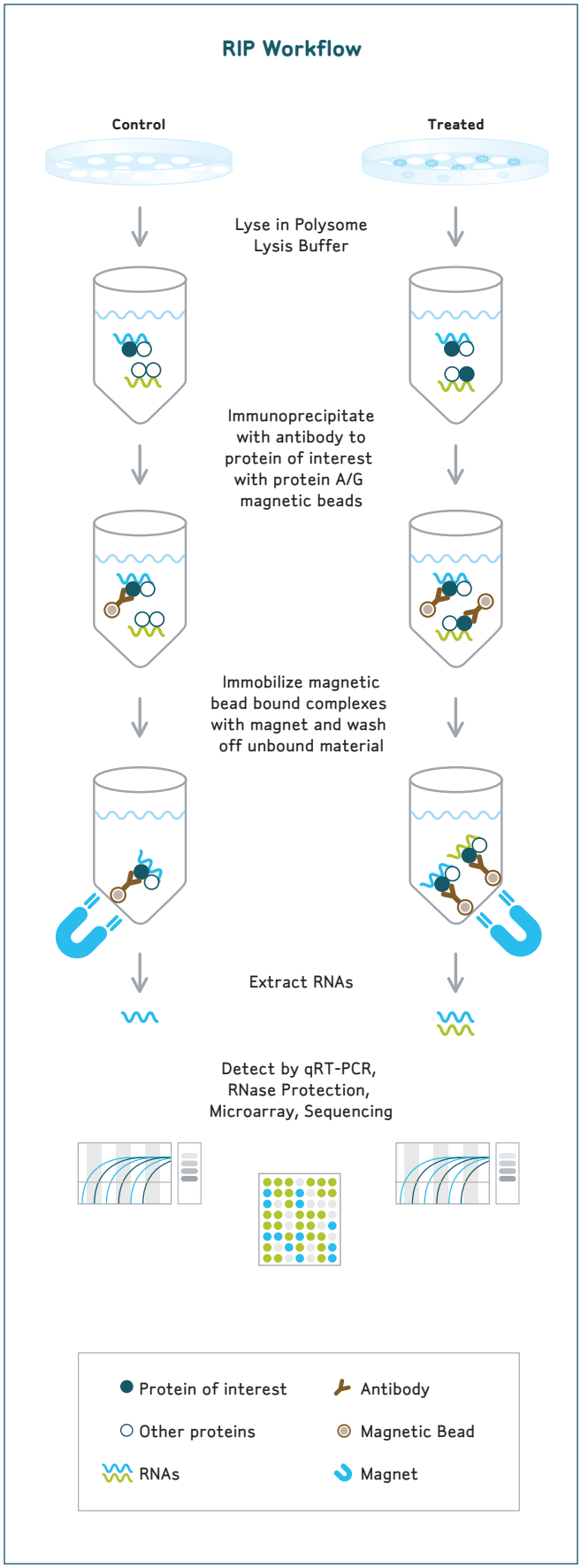
RNA-binding protein immunoprecipitation can be viewed as the RNA analog of the more well-known ChIP application (chromatin immunoprecipitation), which identifies DNA targets of DNA-binding proteins in their cellular context. RIP can be used to identify specific RNA molecules associated with specific 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 (see workflow diagram). 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).

Beginning in the early 1990s, researchers began isolating RNAs from immunoprecipitates and identifying them by Northern blot or reverse-transcription PCR (RT-PCR)[1-3].The post-genomic era has seen the establishment of RIP-Chip protocols for higher throughput identification of RBP-associated RNAs[4,5].

Today, many RNA-focused laboratories regularly employ RIP to study splicing, silencing, and more[6-8]. Millipore's Magna RIP kit makes this powerful technique accessible to everyone, regardless of prior experience with immunoprecipitation- or RNA-based procedures.

References:

1. Selinger, D. *et al.* (1993) *Mol Cell Biol.* **13(3)**: 1353–1362.
2. Tarn, W.Y., *et al.* (1993) *Mol Cell Biol.* **13(3)**: 1883-91.
3. Buckanovich, R.J., *et al.* (1997) *Mol Cell Biol.* **17(6)**: 3194-201.
4. Tenenbaum, S.A., *et al.* (2000). *PNAS.* **97(26)**: 14085-14090.
5. Keene, J.D., *et al.* (2006). *Nature Protocols.* **1(1)**: 302-307.
6. Friend, K., *et al.* (2007) *Mol Cell* **28(2)**: 240-252.
7. Mourelatos, Z. *et al.* (2002) *Genes Dev* **16(6)**: 720-728.
8. Calabrese, J.M., *et al.* (2006) *RNA* **12(12)**: 2092-2102.



Magna RIP RNA-Binding Protein Immunoprecipitation Kits

The Millipore universal RIP kit is fully compatible with a wide range of RIP validated antibodies, and contains all reagents needed for robust, specific enrichment of RBP-associated RNAs.

Description	Reactions	Catalogue No.
Magna RIP Kit	12 reactions	17-700
EZ-Magna RIP Kit, with positive control antibody and primers	12 reactions	17-701
Magna RIP Quad Kit	48 reactions	17-704

Performance of the Magna RIP Kit Control Antibody (qualitative)

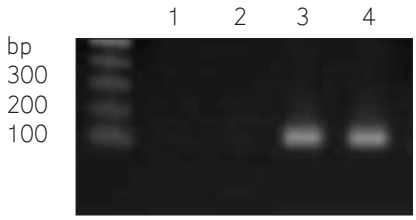


Figure A: RIP was performed using HeLa cell lysate and either anti-SNRNP70 (lane 3) or Normal Rabbit IgG (lane 2) as the immunoprecipitating antibody. Purified RNA was then analyzed by RT-PCR using Control Primers specific for the U1 snRNA. PCR product was observed in the anti-SNRNP70 IP (lane 3). U1 snRNA-specific cDNA was also observed in the 10% Input (lane 4) and not in the "No template" PCR control (lane 1).

PCR Analysis of RIP (quantitative) Performance of the Magna RIP Kit Control Antibody

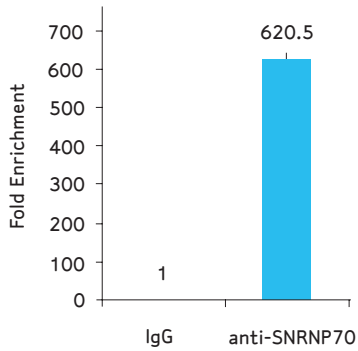


Figure B: RIP was performed using HeLa cell lysate and either anti-SNRNP70 or Normal Rabbit IgG as the immunoprecipitating antibody. Purified RNA was then analyzed by qRT-PCR using Control Primers specific for the U1 snRNA. Only RNA associated with the anti-SNRNP70 antibody was enriched by immunoprecipitation.

RIPAb+ RIP Validated Antibody/Primer Sets

All RIPAb+ antibodies are validated for RNA-binding protein immunoprecipitation. Each RIPAb+ antibody set includes control primers (each lot tested by qPCR) to biologically validate your RIP results by qPCR detection of specific RNAs where possible. The RIPAb+ set also includes a negative control antibody to guarantee specificity of the RIP reaction.

Description	Reactions	Catalogue No.
RIPAb+ PABPC1 *Coming Soon!*	10 reactions	03-101
RIPAb+ HuR *Coming Soon!*	10 reactions	03-102
RIPAb+ SNRNP70 *Coming Soon!*	10 reactions	03-103

More RIPAb+ antibody/primer sets coming soon.
Please visit **www.millipore.com/RIP** for an up-to-date listing.

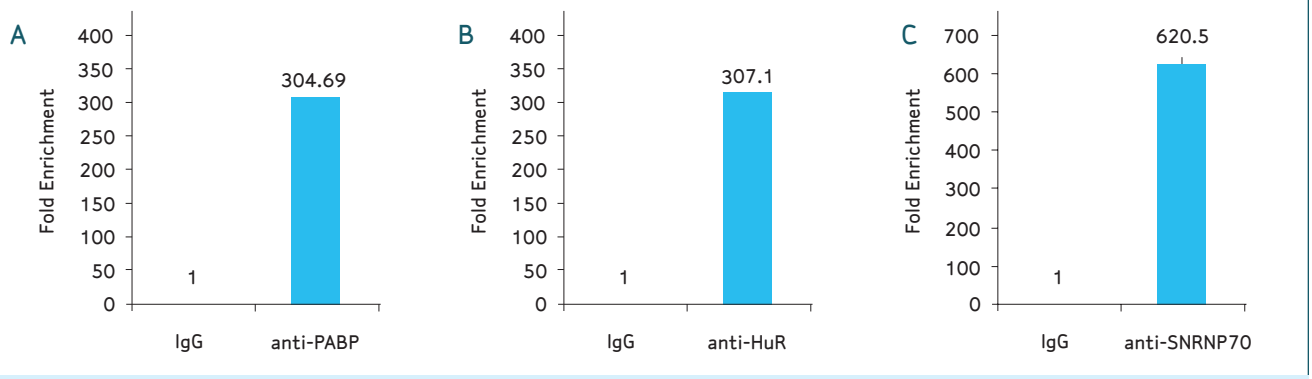


Figure A: RIP Lysate prepared from HeLa cells (2 x 10^7 cell equivalents per IP) were subjected to immunoprecipitation using 5 µg of either a normal mouse IgG, or Anti-PABP antibody and the Magna RIP Kit (Catalogue No. 17-700). Successful immunoprecipitation of PABP-associated RNA was verified by qPCR using Control Primers, Actin B.

Figure B: RIP Lysate prepared from HeLa cells (2 x 10^7 cell equivalents per IP) were subjected to immunoprecipitation using 5 µg of either a normal Rabbit IgG, or Anti-HuR antibody and the Magna RIP Kit (Catalogue No. 17-700). Successful immunoprecipitation of HuR-associated RNA was verified by qPCR using Control Primers, Actin B.

Figure C: RIP Lysate prepared from HeLa cells (2 x 10^7 cell equivalents per IP) were subjected to immunoprecipitation using 5 µg of either a normal rabbit IgG or Anti-SNRNP70 antibody and the Magna RIP Kit (Catalogue No. 17-700). Successful immunoprecipitation of SNRNP70-associated RNA was verified by qPCR using Control Primers, U1 snRNA.

Please refer to the Magna RIP (Catalogue No. 17-700) or EZ-Magna RIP™ (Catalogue No. 17-701) protocol for experimental details.

RNA-Binding Protein (RBP) Antibodies

Millipore also provides a wide selection of antibodies towards a variety of RNA-binding proteins.

Description	Catalogue No.
Anti-4E-BP1, Rabbit Monoclonal	04-321
Anti-Ago1	07-599
Anti-Ago1, clone 6D8.2	04-083
Anti-Ago2	07-590
Anti-Ago2, clone 9E8.2	04-642
anti-Ago2, clone 9E8.2	04-084
Anti-Ago4, clone 5F9.2	05-967
Anti-Ago Family	04-085
Anti-AIRE	09-456
Anti-AKAP 150	07-210
Anti-AKAP 79	07-235
Anti-AKAP 95	06-417
Anti-AUF1	07-260
Anti-BARD1	AB10004
Anti-Bnip3L, internal	AB16507
Anti-BRAF35, clone 4.21	05-641
Anti-CtBP-1	07-306
Anti-CUGBP1, clone 3B1	05-621
Anti-CUGBP2, clone 1H2	04-047
Anti-Dicer1, clone 5D12.2	04-721
Anti-Drosha	07-717
Anti-Ebp1	07-397
Anti-EF1α, clone CBP-KK1	05-235
Anti-EF2 Kinase	07-589
Anti-eIF4E Binding Protein	AB3251
Anti-eIF4E CT, Rabbit Monoclonal	04-347
Anti-ESET/SetDB1	07-1568
Anti-Fragile X Mental Retardation Protein, clone 1C3	MAB2160
Anti-hnRNP A0	07-504
Anti-hnRNP K, clone F45P9C7	04-088
Anti-hnRNP M1-M4, clone 1D8	05-620
Anti-HuB	AB5969
Anti-HuC	AB5829
Anti-HuC	AB15882
Anti-HuD	AB5971
Anti-HuR	AB5831
Anti-HuR	07-1735

Description	Catalogue No.
Anti-HuR	07-468
Anti-Iron Regulatory Protein 1	AB15506
Anti-Iron Regulatory Protein 2	AB15508
Anti-Iron Regulatory Protein 2	MAB5582
Anti-Lin28	07-1385
Anti-MBNL, clone 3A4	04-048
Anti-MDM2	07-575
Anti-MDM2, clone SMP14	MAB3776
Anti-MSI2, clone 1F2	MAB10085
Anti-Musashi	AB5977
Anti-Musashi	AB15648
Anti-Nova-1	07-637
Anti-Nuclear Ribonucleoprotein, clone 58-15	MAB1287
Anti-Nucleolin	05-565
Anti-NXF2	AB15244
Anti-p54nrb/NonO, clone 78-1-C6	05-950
Anti-p68, clone PAb204	05-850
Anti-PABP, clone 10E10	05-847
Anti-PABPC4, clone 6E1.2	MAB11015
Anti-PGC-1	AB3242
Anti-phospho eIF4E (Ser209)	07-823
Anti-phospho-eIF2Bε (Ser539)	07-822
Anti-phospho-eIF-2α (Ser51)	07-760
Anti-phospho-eIF-2α (Ser51), rabbit monoclonal	04-342
Anti-phospho-eIF4G (Ser1108)	07-824
Anti-phospho-hnRNP A0 (Ser84)	07-566
Anti-PUM2, clone 1E10	MAB10104
Anti-QKI-5	AB9904
Anti-QKI-6	AB9906
Anti-QKI-7	AB9908
Anti-RBMS3, clone 1H6	MAB10105
Anti-RNase L, clone 2G5	05-839
Anti-RO52	AB4146
Anti-S100L Protein, clone S100-14	MAB385
Anti-Staufen	AB5781
Anti-TATA-Binding-Protein-Associated Factor II68, a.a. 175-414	MAB3672

For a complete listing of RNA-Binding Protein Antibodies, please visit **www.millipore.com/RIP**.

Increase the Efficiency & Performance of Your RNA Experiments with Nuclease-Free Water

BioPak Point-of-Use Ultrafilter

Final purification step at the ultrapure water point-of-delivery provides pyrogen- and nuclease-free water at a high flow rate when you need it.

KEY BENEFITS:

- Direct connection to all Millipore Type I water systems
- Pyrogen-free water (< 0.001 EU / mL) production
- RNase-free water (< 0.01 ng / mL) and DNase-free water (< 4 pg / µL) production
- Safe method that eliminates the need to treat water with DEPC
- Bacteria-free water (< 1 cfu / mL) production
- Warranty of results within specifications for a minimum of 90 days usage
- Maintenance-free

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