

Epigenetics

Millipore's Magna RIP™ (RNA-Binding Protein Immunoprecipitation) Kit and RIPAb+ Validated Reagents Immunological Tools to Explore Post-Transcriptional Regulation of RNAs

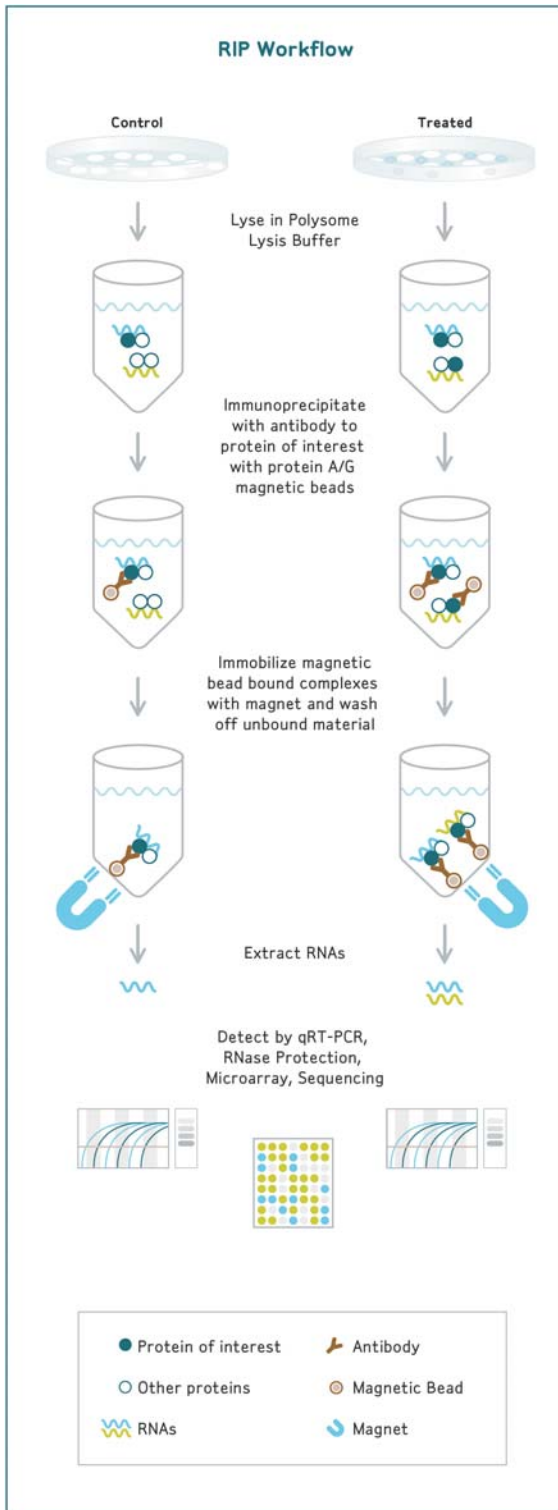


Figure 1. RIP Assay Overview
After the lysis of the cells in polysome lysis buffer RNA-Protein complexes are immunoprecipitated with magnetic protein A/B beads. RNAs are isolated from the complex and subjected to qRT-PCR, RNase protection assay, Micro array, or sequencing.

Abstract

Gene expression plays a critical role in complex cellular processes such as development, differentiation, and cellular response to environmental changes. In addition to transcriptional regulation of gene expression by transcription factors, cells utilize post-transcriptional regulatory mechanisms. One such mechanism involves use of certain RNA binding proteins (RBPs) to temporally and coordinately regulate the rate of mRNA translation of functionally related gene products. While the regulation of gene expression by transcription factors has been well studied over time, the post-transcriptional regulation of mRNAs by RBPs and non-coding RNAs is a fairly nascent field that remains to be thoroughly explored.

RNA-Binding protein immunoprecipitation (RIP) is the RNA analog of the more well-known ChIP application (chromatin immunoprecipitation), which identifies DNA targets of DNA-binding proteins in an *in-vivo* cellular context. RIP can be used to identify specific RNA molecules (of many types) associated with specific nuclear or cytoplasmic binding proteins. These experiments involve immunoprecipitation of endogenously formed complexes of RNA binding proteins and co-isolation of any RNA species associated with that RNA binding protein. Purification of these RNA species allows interrogation and identification of mRNAs (and potentially non-coding RNAs associated with them) and can be directly subjected to a variety of applications including quantitative RT-PCR, microarray analysis (RIP-chip) and “deep-sequencing” or 2nd-generation sequencing based platform (RIP-Seq).

To enable the exploration of these RNA binding events, we have focused on optimizing the methods and developing a variety of RIP validated reagents. The Millipore universal RIP immunoprecipitation kit allows researchers to perform RIP experiments using a variety of RIP validated antibodies. Additionally, we offer RIP validated antibodies available separately in kit type format including controls useful for validation of RIP experiments, designated RIPAb+ kits. This new level of antibody application testing and lot to lot quality control validation will provide confidence in end users’ RIP experiments and allow comparison of RIP profiles to other published RIP results including ENCODE datasets.

MILLIPORE Magna RIP™ Immunoprecipitation Kit (#17-700)



Kit Components

- | | | | |
|---|------------------------------------|----|---------------------------|
| 1 | Magnetic Protein A/Protein G beads | 9 | 10% SDS |
| 2 | Immunoprecipitation Buffer | 10 | Salt Solution I |
| 3 | Polysome Lysis Buffer | 11 | Salt Solution II |
| 4 | 0.1 M DTT | 12 | Precipitate Enhancer |
| 5 | 0.5 M EDTA | 13 | Positive Control Antibody |
| 6 | Protease Inhibitor Cocktail | 14 | Negative Control Antibody |
| 7 | RNAse Inhibitor | 15 | Positive Control Primers |
| 8 | Proteinase K | | |

Figure 2. The Magna RIP kit contains the buffer and reagents required to perform a successful RIP experiment from mammalian cells. Importantly, the kit also contains essential controls such as positive and negative control antibodies and Control Primers to ensure that the user has successfully adopted the RIP protocol.

Use of the U1 70K (SNRNP70) Control Antibody in RIP

U170K (SNRNP70) is an RNA-binding protein, which specifically binds to the loop I region of U1-snRNA. Thus the RNA-protein complex immunoprecipitated with an antibody directed against U170K is enriched for the U1-snRNA, where as immunoprecipitation with normal IgG should not result in significant U1-snRNA enrichment. We include anti-U170K as a positive control for the RIP assay. This antibody efficiently and superficially enrich the U1-snRNA.

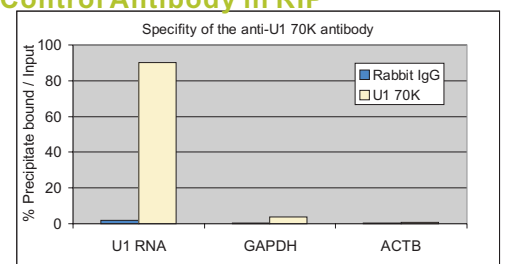


Figure 3. Specificity of the anti-U1 70K antibody. The anti-U1 70K antibody that are considered for RIP validation are initially characterized by RIP assay and qRT-PCR using U1 RNA, GAPDH and ACTB primers to confirm specificity.



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RIPAb Validated Antibodies

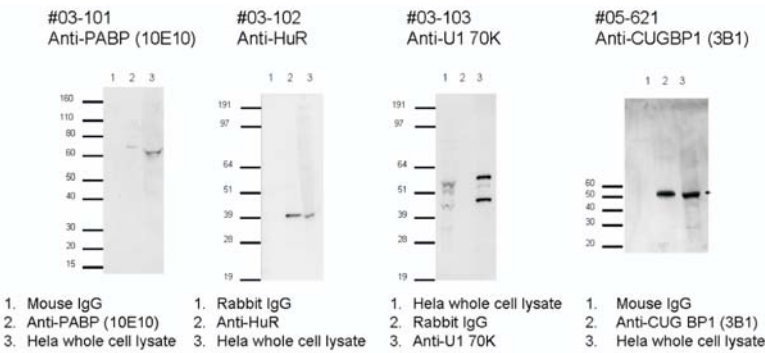


Figure 4. Polyclonal and monoclonal antibodies that are considered for RIP validation are initially characterized by Immunoprecipitations under RIP conditions followed by immunoblotting.

Conclusions

- Employing magnetic beads technology and optimized buffers we have developed the Magna RIP™ RNA-binding protein Immunoprecipitation kits. These kits will allow researchers to perform the RIP experiments faster and easier compares to traditional methods.
- We also offer variety of RIPAb antibodies (validated the ability to immunoprecipitate target RBP-complex) and RIPAb+ antibodies (RIPAb antibodies those are confirmed the presence of specific RNAs in the complex by qRT-PCR). The RIPAb+ kit product line includes the RIPAb antibody, Negative Control antibodies and qPCR primer sets that are used to confirm the presence of specific RNAs in the complex.

RIPAb+ Antibody Kits

Following initial RIPAb characterization, antibody candidates for RIPAb+ products are subsequently confirmed the presence of specific RNAs in a complex by qRT-PCR. RIPAb+ Kit product line includes RIPAb+ antibody, negative control antibody and RT-PCR/qPCR primer sets that are used to confirm presence of specific RNAs in a complex.

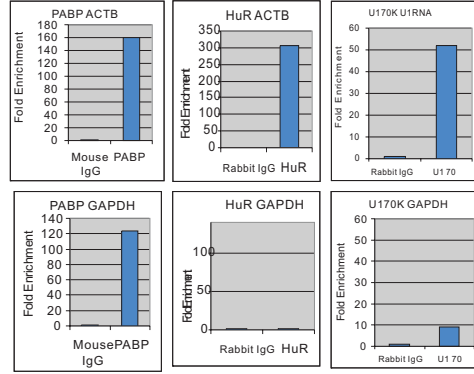


Figure 5. Each lot of RIPAb+ antibody is tested in a RIP qRT-PCR assay using HeLa Lysate and GAPDH and ACTB primers (PABP, HuR) or U1-RNA and GAPDH primers (U1 70K). RIP assays are performed using Magna RIP kit, and Fold Enrichment relative to negative control is derived from a standard curve of total Input for each assay.

#03-101 Anti-PABP (10E10)
#03-102 Anti-HuR
#03-103 Anti-U170K

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NEW Magna RIP RNA-Binding Protein Immunoprecipitation Kits

做 RNA-Binding Protein 研究的人有福了!

Millipore 提供您簡單又方便的 all-in-one kit

- ☑ 採用磁珠式 Protein A/G 快速分離 Target RNA-Binding Protein
- ☑ 配方專為 binding nucleic acid-protein complexes 設計, 不需要再花時間測試實驗條件
- ☑ 試劑組含 RNase inhibitors & RNase-free reagents, 不用擔心 RNA 在實驗的過程中被分解
- ☑ 試劑組含 Negative control
- ☑ EZ-Magna RIP Kit 含 positive control antibody & primers

Ordering Information

#17-700	Magna RIP Kit	12 reactions
#17-704	Magna RIP Quad Kit	48 reactions
#17-701	EZ-Magna RIP Kit	12 reactions

購買任一 RIP Kit, 立即享有 **RNA-Binding Protein Ab 半價優惠**

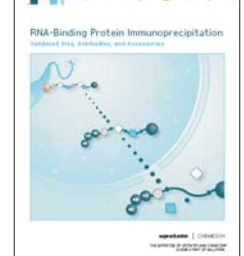
(活動至 2009/12/31 止)

Why RBP is so important?

- a key role in **post-transcriptional regulation of gene expression**
- Involved in every step of **mRNA metabolism**
- Potential to affect the manner and rate of **protein synthesis**

想了解更多 RBP 嗎?

歡迎來電索取最新目錄



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