Validated RBP antibodies: Enliven your RIP protocols

RNA-binding protein antibodies validated for RNA immunoprecipitation.

RNA-binding proteins (RBPs) recognize specific RNA sequences or structures and control many molecular functions, including transcription, splicing, 5′ RNA capping, polyadenylation, mRNA export, ribosome assembly, translation, and RNA decay. These diverse functions allow RBPs to wield exquisite control over gene expression that is either dependent on or independent of transcription. Because these proteins play significant roles in regulating gene expression, identifying which RNAs a specific RBP associates with can provide powerful information for dissecting downstream functions. Similar to chromatin immunoprecipitation (ChIP), RNA immunoprecipitation (RIP) often starts with the crosslinking of live cells, followed by immunoprecipitation of RNA–protein complexes using a specific RBP antibody, and recovery and identification of the RNA sequences [1]. Thermo Fisher Scientific has instituted validation* programs for Invitrogen™ RBP antibodies that use RIP and RIP-western (RIP followed by western blotting to determine the protein immunoprecipitated). Here we highlight a few targets in our rapidly growing RBP antibody portfolio.

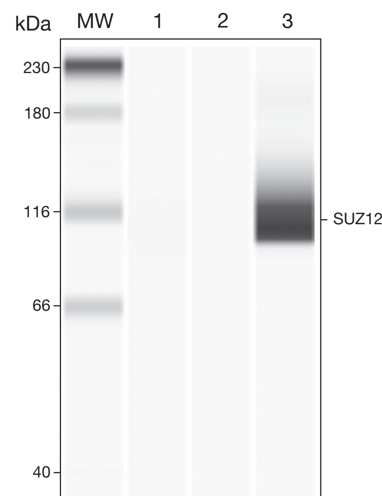

Figure 1. Immunoprecipitation of SUZ12 in K562 cells. Antigen–antibody complexes were formed by incubating ~500 µg whole cell lysate with 5–10 µL Invitrogen™ SUZ12 Recombinant Rabbit Monoclonal Antibody (clone 7H26L21, Cat. No. 702490) and rotating for 60 min at room temperature. The immune complexes were captured on 625 µg Invitrogen™ Dynabeads™ M-280 Sheep Anti–Rabbit IgG superparamagnetic beads (Cat. No. 11203D) and washed extensively. They were then eluted and analyzed on the ProteinSimple™ Simple Western™ system (Bio-Techne) with the same antibody used for immunoprecipitation (IP) at a dilution of 1:25, followed by a 1:100 dilution of secondary antibody: lane 1, the input; lane 2, no antibody used for IP; lane 3, target-specific antibody used for IP. Data courtesy of the GW Yeo lab, University of California San Diego, as part of the ENCODE project.

RNA binding by the PRC2 complex

The polycomb repressive complex 2 (PRC2), which has histone methyltransferase activity, plays a critical role in gene regulation by controlling the trimethylation of histone H3 on lysine 27 (H3K27me3)—one of the hallmarks of transcriptionally silent chromatin. Dysregulation of PRC2 has been implicated in several types of cancer, and PRC2 subunits have long been targets for therapeutics. Although PRC2 subunits have been shown to bind to numerous types of RNA, the complex's function is still rather elusive [2]. For example, PRC2 interacts with many nascent RNAs, leading researchers to question the function of this promiscuous binding.

The "junk-mail model" suggests that promiscuous binding of PRC2 serves as a checkpoint—PRC2 identifies target genes that have escaped repression by binding to the newly transcribed RNA and then scanning the local chromatin for H3K27me3, which signals that the gene should be silenced. Alternatively, the "antagonistic model" is based on competition of RNA and chromatin for the binding of PRC2. For highly expressed genes, PRC2 binding shifts to the nascent RNA; for transcriptionally silent genes, RNA levels are low so PRC2 binding shifts to chromatin. Furthermore, it has been proposed that PRC2 may prefer certain RNA secondary structures—such as RNA G-quadruplexes [3]. The ability to analyze which RNAs bind the PRC2 complex is essential for a better understanding of the biological consequences of these interactions. Figure 1 shows that SUZ12, a core component of PRC2, can be immunoprecipitated using a recombinant monoclonal SUZ12 antibody and then analyzed by RIP-western. RIP and other experiments that examine RNA–protein interactions will help to clarify the function of PRC2 binding to RNA.

mRNA binding by IMPs

The highly conserved IMP family of mRNA-binding proteins, also known as insulin-like growth factor 2 mRNA binding proteins (IGF2BPs), regulate RNA localization, translation, and stability, and associate with over 1,000 target mRNAs [4]. IMP1 (IGF2BP1) and IMP3 (IGF2BP3) are primarily expressed during embryogenesis, although re-expressed in various tumor tissues [5,6]. IMP2 (IGFBP2) has been observed during development and in adult mice and has been shown to be expressed at elevated levels in cancer cells [7]. Although their roles are still being investigated, the IMPs selectively bind to and regulate their RNA targets. Dysregulated binding due to aberrant expression is associated with different types of cancer.

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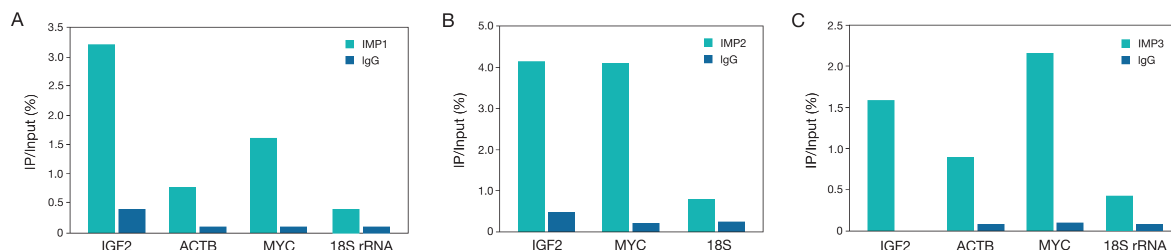

Figure 2. Detection of IMP binding to specific RNAs using RIP. The binding of endogenous IMPs to specific RNAs was determined by RNA immunoprecipitation (RIP) using Invitrogen™ recombinant polyclonal antibodies to **(A)** IMP1 (IGF2BP1) (Cat. No. 712138), **(B)** IMP2 (IGF2BP2) (Cat. No. 712137), and **(C)** IMP3 (IGF2BP3) (Cat. No. 712139) on clarified whole cell lysate from 2 x 10^6 HepG2 cells. In all experiments, normal rabbit IgG was used as a negative IP control. Immunoprecipitated RNA was purified using the Invitrogen™ RiboPure™ RNA Purification Kit (Cat. No. AM1924) and analyzed by RT-qPCR using the Applied Biosystems™ *Power* SYBR™ Green RNA-to-C_T™ 1-Step Kit (Cat. No. 4389986) with primer pairs for IGF2, MYC, and ACTB mRNA (positive), and 18S rRNA (negative). Data are presented as the fraction of RNA immunoprecipitated (IP) normalized to the total amount of RNA used for immunoprecipitation (input).

Several target mRNAs were used with RIP to explore the specificity of recombinant polyclonal IMP antibodies in HepG2 cells (Figure 2), a human liver cancer cell line. The IGF2 transcript is a target for all three IMPs, although IMP1 inhibits translation, whereas IMP2 and IMP3 enhance translation. ACTB and MYC transcripts have been described as targets for IMP1, but enrichment of these mRNAs was seen for all three IMPs in HepG2 cells, which may be related to the role of IMP2 and IMP3 in liver cancer. IMPs have not been known to associate with 18S rRNA, and our RIP data are consistent with this observation.

Techniques for examining RNA–protein interactions

RIP is an excellent technique for identifying RNAs within an RNA–protein complex but may not be well suited for identifying direct RNA–protein interactions. A modification of RIP—the crosslinking and immunoprecipitation technique (CLIP)—uses UV crosslinking and stringent conditions to isolate only the specific RBP of interest and RNA species to which it is bound, and not other proteins and RNA contained within the complex. Several refinements have been developed, including the enhanced CLIP technique (eCLIP) shown in Figure 3 [8]. Immunoprecipitation with eIF4E and hnRNP K monoclonal antibodies demonstrate that they bind at different locations in TRA2A, as shown by shifts in peak density.

Find RIP-validated antibodies for your research

RIP, CLIP, and other immunoprecipitation methods will be essential for mapping the binding sites of RBPs across the transcriptome. Find specific, RIP-validated antibodies at **thermofisher.com/antibodies**. ■

*The use or any variation of the word "validation" refers only to research use antibodies that were subject to functional testing to confirm that the antibody can be used with the research techniques indicated. It does not ensure that the product(s) was validated for clinical or diagnostic uses.

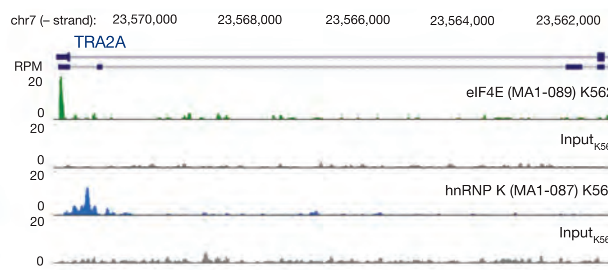

Figure 3. eIF4E and hnRNP K read density in reads per million (RPM). Shown are eCLIP with input controls in K562 cells at TRA2A. Invitrogen™ eIF4E Monoclonal Antibody (clone 5D11, Cat. No. MA1-089) and Invitrogen™ hnRNP K Monoclonal Antibody (clone F45 P9 C7, Cat. No. MA1-087) were used for the immunoprecipitation (see experimental details in [8]). Data courtesy of the GW Yeo lab, University of California San Diego, as part of the ENCODE project.

References

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Product	Quantity	Cat. No.
Dynabeads™ M-280 Sheep Anti-Rabbit IgG	2 mL	11203D
eIF4E Monoclonal Antibody (clone 5D11)	100 µg	MA1-089
hnRNP K Monoclonal Antibody (clone F45 P9 C7)	100 µg	MA1-087
IGF2BP1 Recombinant Polyclonal Antibody (clone 18HCLC)	100 µg	712138
IGF2BP2 Recombinant Polyclonal Antibody (clone 1HCLC)	100 µg	712137
IGF2BP3 Recombinant Polyclonal Antibody (clone 6HCLC)	100 µg	712139
Power SYBR™ Green RNA-to-C_T™ 1-Step Kit	100 reactions	4389986
RiboPure™ RNA Purification Kit	50 preps	AM1924
SUZ12 Recombinant Rabbit Monoclonal Antibody (clone 7H26L21)	100 µg	702490

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