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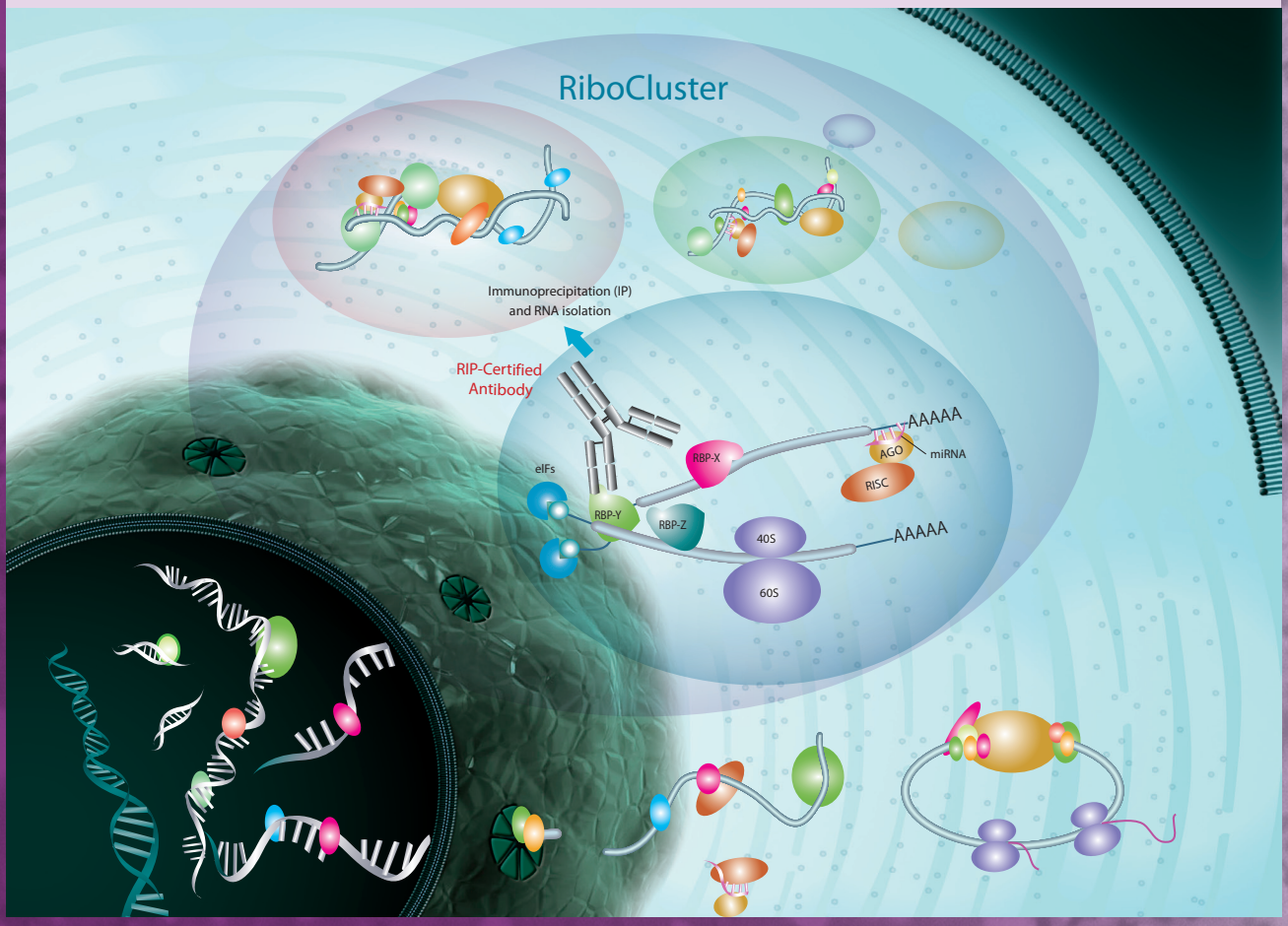
Novel Tools for Post-Transcriptional Regulation of Gene Expression Research



Recent studies suggest that certain disease- or function-related mRNAs or microRNAs (miRNAs) bind to RNA-binding proteins (RBPs), forming clusters which are termed here as RiboClusters. RBPs play an important role in post-transcriptional regulation of gene expression at various steps such as splicing, nuclear export subcellular localization, mRNA stability and translation. RiboCluster ProfilerTM is an optimized unique tool which enables customers to extensively analyze the certain disease- or function-related genes.

RIP-Chip technology is patented by Ribonomics, Inc. (US patent number 6,635,422 and 7,504,210)
MBL has the world-wide, exclusive license of Ribonomics' patents to develop, manufacture and sell RIP-Chip related products.

RiboCluster Profiler™

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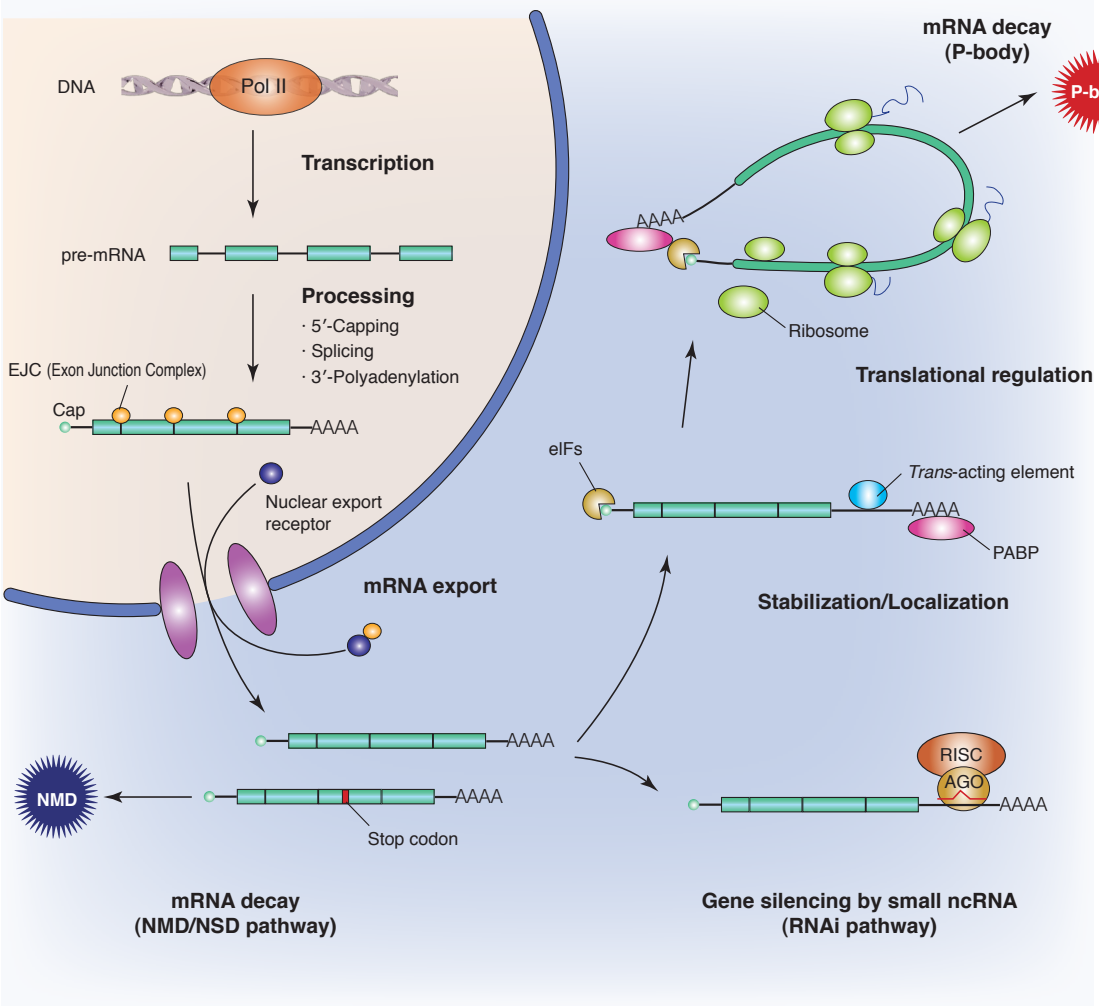
Post-transcriptional regulation mechanism



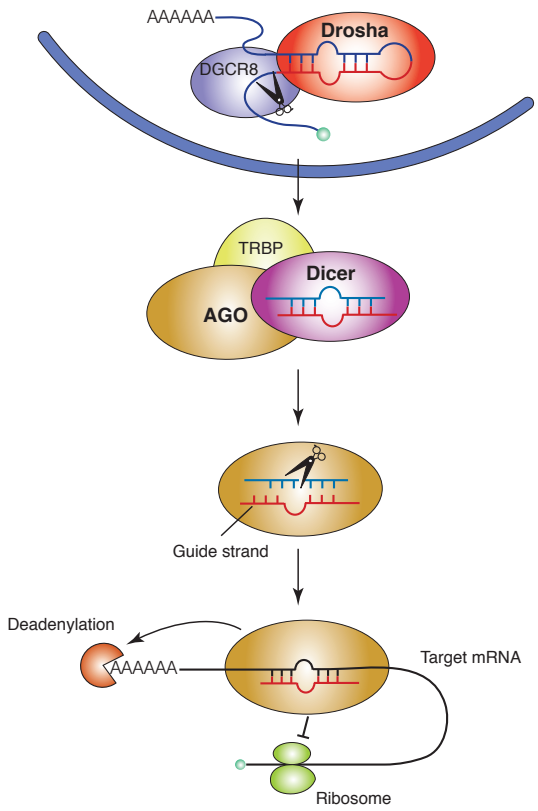
In eukaryotic cells, there are a lot of regulatory mechanisms, which control quality of mRNA in various steps from processing of precursor mRNA (pre-mRNA) in nucleus to translation into protein through cytoplasmic ribosome. Among these processes, RBPs play a primary role in regulating the behavior of the functionally related genes by forming the ribonucleoprotein (RNP) clusters.

An emerging interest in RNA world

A non-coding RNA (ncRNA) is a functional RNA molecule that is not translated into a protein, whereas a mRNA is translated into a protein. The ncRNAs include highly abundant and functionally important RNAs such as transfer RNA (tRNA), ribosomal RNA (rRNA), as well as small ncRNA and long ncRNA. The main classes of small ncRNAs are miRNA, short interfering RNA (siRNA), and PIWI-interacting RNA (piRNA), and all of them play an important role in RNA interference (RNAi) pathway. Many reports suggest that long ncRNAs are involved in structure of functional nuclear domain.

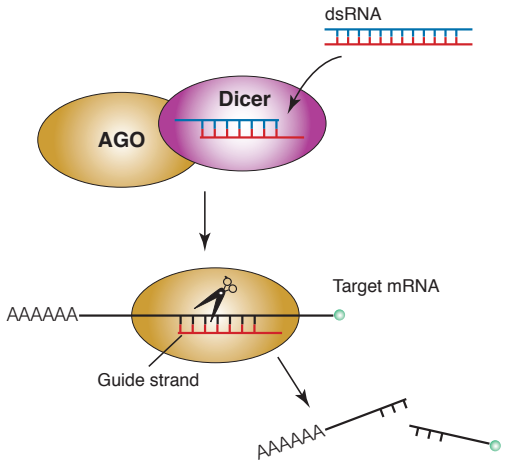
Non-coding RNA		Length (nt)	Species	Function
Ribosomal RNA (rRNA)		120~4700	All	Translation
Transfer RNA (tRNA)		70~100	All	Translation
Small nuclear RNA (snRNA)		70~350	Eukaryote	Splicing, mRNA processing
Small nucleolar RNA (snoRNA)		70~300	Eukaryote, archaea	RNA modification, rRNA processing
miRNA	Small ncRNA	21~25	Eukaryote	Translational regulation
siRNA	Small ncRNA	21~25	Eukaryote	Protection against viral infection
piRNA	Small ncRNA	24~30	Eukaryote	Genome stabilization
Long ncRNA		several hundreds~ several hundred thousands	Eukaryote	Transcription, splicing, transport regulation

miRNA pathway



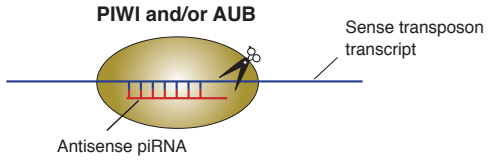
MicroRNA (miRNA) is mainly transcribed from endogenous miRNA gene as a primary transcript (pri-miRNA), which contains 65-70-nucleotide stem-loop structure. The hairpin structure of pri-miRNA is excised by Drosha, an RNase III-family enzyme, to yield a precursor miRNA (pre-miRNA). Nuclear export factors, such as Exportin-5, transport the pre-miRNA from nucleus to cytoplasm where Dicer, another RNase III enzyme, cleaves the pre-miRNA to generate a miRNA-miRNA* duplex of 21-25 nucleotides in length. The duplex is unwound and one strand (guide strand) is loaded onto an Argonaute (AGO) protein. The guide strand directs the AGO to target mRNAs that contain sequence complementarity to the guide strand in the 3'-untranslated regions (3'-UTRs) to suppress translation of the target mRNAs.

siRNA pathway



siRNA is thought to function as a defense mechanism against RNA virus. Dicer has RNase III activity and produces a siRNA duplex of 21-25 nucleotide length from long double-stranded RNA (dsRNA) in the cytoplasm. The guide strand of the siRNA duplex is loaded onto AGO, a component of RNA-induced silencing complex (RISC), where it gets attached to complementary mRNA sequences and silences those transcripts. The unwound passenger strand is removed from the RISC and degraded.

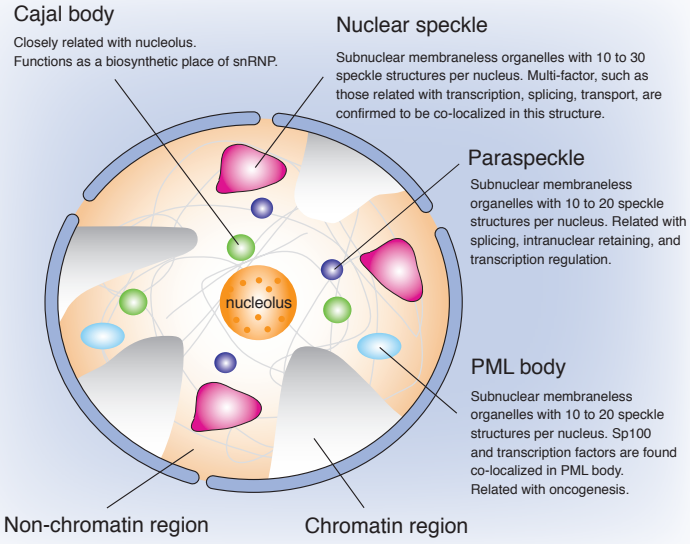
piRNA pathway



PIWI-interacting RNAs (piRNAs) are derived from single-stranded transposon transcripts. piRNA fragments are produced by the poorly understood “primary processing pathway”, which does not require RNase III activity. piRNA is loaded onto PIWI, a family of protein only expressed in germ cells, where it induces the cleavage of complementary transposon transcripts.

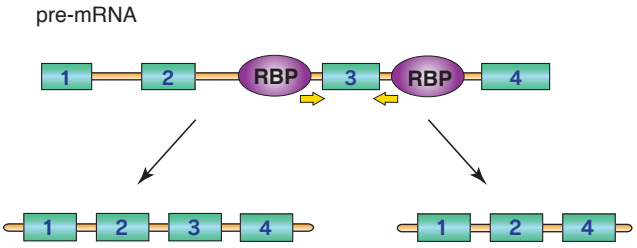
mRNA-like ncRNA and intranuclear functional domain

Recent transcriptome analyses have revealed that transcription occurs at more than 90% of human genomic DNA regions, and more than 95% of transcripts are predicted to be ncRNAs. That is to say, most of the transcribed RNAs are ncRNAs. Although annotation of ncRNAs has been started in several species, the functions of most of them are still unknown. Some of them have been confirmed to be mRNA-like ncRNAs that undergo post-transcriptional processing, such as 5'-capping, splicing and 3'-polyadenylation, without being translated. As the study on RNA became an active area of research, it has been unveiled little by little that the most of the mRNA-like ncRNAs reside in the nucleus and function as an essential structural determinant of nuclear functional domains. ncRNAs and RBPs are aligned and regulated in a spatiotemporally specific manner in each nuclear functional domain. These findings support the noticeable concept, ‘RiboCluster’, propounded by MBL.



Splicing

Splicing removes the introns in pre-mRNA transcribed from genome and joins the exons together. Great varieties of RBPs are involved in inducing the alternative splicing. The resulting different mRNAs may be translated into different proteins in isoforms and thus increase the diversity of the protein.



RIP-Certified Antibody

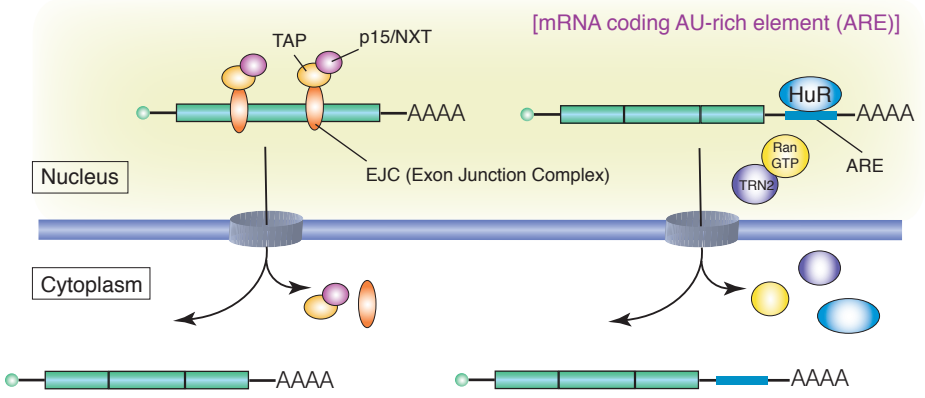
Code No.	Product name (Gene name)	Clone Application	Isotype Species reactivity	Size	Function, localization, etc
RN045P	anti-SLBP (HBP)	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo,Rat,Hm	200 µL	SLBP binds to stem-loop structure in 3' end of histone pre-mRNA and is related with processing, transport, translation, degradation and cell cycle regulation.
RN019P	anti-HNRNPK (HNRPK)	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo,Rat	200 µL	HNRNPK, one of the components of hnRNP complex, shuttles between nucleus and cytoplasm. It is related with splicing, transport and translational regulation of mRNA.
RN014P	anti-TIA1	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo,Rat,Hm	200 µL	TIA1 shuttles between nucleus and cytoplasm. It is related with alternative splicing of genes including Fas and FGFR2.
RN015P	anti-YBX1	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo,Rat,Hm	200 µL	YBX1, a member of cold shock protein family, is one of the main components of cytoplasmic mRNP particles. Some reports indicate it is related with splicing of mRNA.
RN011P	anti-PTBP1 (hnRNPI)	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu	200 µL	PTBP1 is related with alternative splicing and negatively regulates the splice site via binding with intronic cluster of pre-mRNA.
RN021P	anti-KHDRBS1 (p62, SAM68)	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo,Rat	200 µL	KHDRBS1, a DNA/RNA binding protein, affects inclusion of CD44 exon v5 via regulating alternative splicing. It is related with export of HIV RNA as well.
RN041P	anti-KHDRBS2 (SLM1)	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo,Rat,Hm	200 µL	SLM1 is phosphorylated during mitosis and binds to proteins containing SH2 and SH3 domains. It is related with alternative splicing via regulating the selection and exon inclusion of splice site.

RBP Antibody

Code No.	Product name (Gene name)	Clone Application	Isotype Species reactivity	Size	Function, localization, etc
RN047PW	anti-PTBP2	Polyclonal WB,IPP	rab Ig (aff.) Hu	100 µL	PTBP2, specifically expressed in brain, is involved in alternative splicing via binding to pre-mRNA intronic cluster.
RN002MW	anti-CUGBP1 (CELF1)	3B1 WB,IPP	mo IgG1 κ Hu,Mo,Rat	100 µL	CUGBP1 binds to mRNAs of c-jun and TNFRSF1B through their GU-rich element and regulate their alternative splicing and degradation.
RN034PW	anti-CUGBP1 (CELF1)	Polyclonal WB	rab Ig (aff.) Hu,Mo,Rat,Hm	100 µL	It has been reported that CUGBP1 plays a role in the pathogenesis of the trinucleotide expansion disease, namely, myotonic dystrophy (DM1).
RN035PW	anti-CUGBP2 (CELF2)	Polyclonal WB	rab Ig (aff.) Hu,Mo,Rat,Hm	100 µL	CUGBP2 is not expressed only in muscle but is ubiquitous. It stabilizes COX2 mRNA via binding to 3'-UTR. It induces apoptosis in tumor cells via binding with 3'-UTR of Mcl-1 mRNA.
RN042PW	anti-MBNL1	Polyclonal WB	rab Ig (aff.) Hu,Mo,Rat,Hm	100 µL	Expanded CUG/CCUG repeats and changes of binding situation with MBNL1 may lead to myotonic dystrophy (DM1).
RN043PW	anti-NOVA1	Polyclonal WB	rab Ig (aff.) Hu	100 µL	NOVA1 is a neuron specific splicing factor. It is discovered from the serum of a patient with POMA, an autoimmune disease.
RN044PW	anti-NOVA2 (ANOVA)	Polyclonal WB,IPP	rab Ig (aff.) Hu,Rat	100 µL	NOVA2 serves as a neuron specific alternative splicing factor via binding to YCAY cluster of pre-mRNA.
RN046PW	anti-SYNCRIP (NSAP1, HNRPQ)	Polyclonal WB,IPP	rab Ig (aff.) Hu,Mo,Rat,Hm	100 µL	SYNCRIP, one of the components of spliceosome, is related with efficient splicing of pre-mRNA.

Nuclear export

Once transcribed in nucleus, mRNAs undergo a series of processing (5'-capping, splicing and 3'-polyadenylation) followed by export to cytoplasm mediated by proteins including TAP, RanGTP and RBPs. The RNA export from nucleus to cytoplasm is strictly regulated. During the export process, RBP also plays a primary role for quality control of mRNAs.

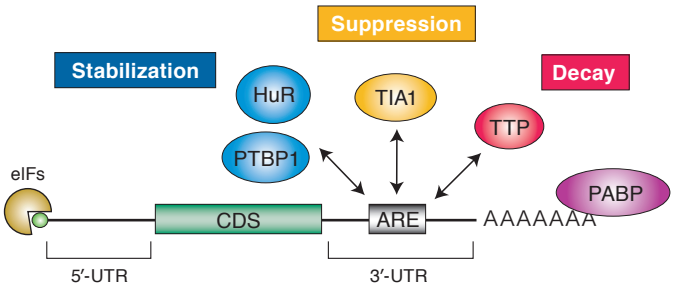


RIP-Certified Antibody

Code No.	Product name (Gene name)	Clone Application	Isotype Species reactivity	Size	Function, localization, etc
RN001P	anti-EIF4E	Polyclonal	rab Ig (aff.)	200 µL	EIF4E, one of the translational initiation factors, binds to m^7G cap structure. EIF4E is also related with transport of mRNA from nucleus to cytoplasm.
		WB,IPP,RIP	Hu,Mo,Rat,Hm		
RN004P	anti-ELAVL1	Polyclonal	rab Ig (aff.)	200 µL	ELAVL1/HuR is ubiquitously expressed and binds with both poly(A) and AU-rich element. It shuttles between nucleus and cytoplasm and may associate with transport of mRNA.
	(HuR)	WB,IPP,RIP	Hu,Mo,Rat,Hm		
RN001M	anti-IGF2BP1	6H6	mo IgG2a κ	200 µL	Together with FMRP, IGF2BP1 binds to β-actin mRNA and transports it from nucleus to cytoplasm.
	(IMP1, ZBP1)	WB,IPP,RIP	Hu,Mo		
RN007P	anti-IGF2BP1	Polyclonal	rab Ig (aff.)	200 µL	IGF2BP1 binding inhibits the initiation of translation, whereas in the destination of cytoplasm, IGF2BP1 is released from mRNA by phosphorylation to initiate the translation.
	(IMP1, ZBP1)	WB,IPP,RIP	Hu,Mo		
RN008P	anti-IGF2BP2	Polyclonal	rab Ig (aff.)	200 µL	IGF2BP2/IMP2 binds to UTR region of IGF2 leader 3 mRNA, which is related with growth and proliferation, and regulates their translation.
	(IMP2)	WB,IPP,RIP	Hu,Mo,Rat		
RN009P	anti-IGF2BP3	Polyclonal	rab Ig (aff.)	200 µL	IGF2BP3/IMP3 binds to 5'-UTR region of IGF2 leader 3 mRNA, which is related with growth and proliferation, and regulates their translation.
	(IMP3)	WB,IPP,RIP	Hu,Mo		
RN045P	anti-SLBP	Polyclonal	rab Ig (aff.)	200 µL	SLBP binds to stem-loop structure in 3' end of histone pre-mRNA and is related with processing, transport, translation, degradation and cell cycle regulation.
	(HBP)	WB,IPP,RIP	Hu,Mo,Rat,Hm		
RN019P	anti-HNRNPK	Polyclonal	rab Ig (aff.)	200 µL	HNRNPK shuttles between nucleus and cytoplasm. Phosphorylation of hnRNPK leads to cytoplasmic accumulation and is important for cell migration and metastasis.
	(HNRPK)	WB,IPP,RIP	Hu,Mo,Rat		
RN021P	anti-KHDRBS1	Polyclonal	rab Ig (aff.)	200 µL	KHDRBS1, a DNA/RNA binding protein, affects inclusion of CD44 exon v5 via regulating alternative splicing. It is related with export of HIV RNA as well.
	(p62, SAM68)	WB,IPP,RIP	Hu,Mo,Rat		
RN016P	anti-FMR1	Polyclonal	rab Ig (aff.)	200 µL	FMR1 is necessary for morphogenesis of nerve system and strongly binds with poly(G) sequence. The expansion of CGG repeats within the FMR1 gene is found in Fragile X syndrome patients.
	(FMRP)	WB,IPP,RIP	Hu,Mo,Rat		
RN017P	anti-FXR1	Polyclonal	rab Ig (aff.)	200 µL	FXR1, known as a paralogue of FMR1, shuttles between nucleus and cytoplasm, and binds with 60S subunit of polysome. It may relate with AGO protein as well.
		WB,IPP,RIP	Hu,Mo,Rat		
RN018P	anti-FXR2	Polyclonal	rab Ig (aff.)	200 µL	FXR2, functionally related with FMR1 and FXR1, shares high homology with FXR1. They include binding with RNA and polysome, and shuttling between the nucleus and cytoplasm.
		WB,IPP,RIP	Hu,Mo,Rat		
RN012P	anti-STAU1	Polyclonal	rab Ig (aff.)	200 µL	STAU-1 forms RNA granule in neuron. It is related with transport and sublocalization of mRNA via binding with dsRNA and with microtubule.
		WB,IPP,RIP	Hu		
RN013P	anti-STAU2	Polyclonal	rab Ig (aff.)	200 µL	STAU2 is highly homologous to STAU1. It shuttles between nucleus and cytoplasm, however, the function in detail is unknown.
		WB,IPP,RIP	Hu		
RN020P	anti-ILF3	Polyclonal	rab Ig (aff.)	200 µL	ILF3 binds to dsRNA. It stabilizes mRNA via binding with AU-rich element of 3'-UTR of IL-2 mRNA, and is related with transport of mRNA from nucleus to cytoplasm.
	(NF90)	WB,IPP,RIP	Hu		

Species reactivity : Hu: Human, Mo: Mouse, Rat: Rat, Hm: Hamster Application : WB: Western blotting, IPP: Immunoprecipitation, RIP: RIP-assay

Regulation of stability and decay



An AU-rich element (ARE) is a region with frequent adenine (A) and uridine (U) nucleotides mainly in the 3'-UTR of a mRNA. Binding of RBPs that have endonuclease activity to this region induces its degradation. Early response genes, which response to a wide range of external stimuli, including oncogenes and cytokines, have relatively short half-lives because of the frequent AREs in theses RNAs. A certain type of RBP, including HuR, binds to ARE and regulates the stability of its target RNA by inhibiting the access of its endonuclease. Similar to AREs, many *cis*-elements have been found to be associated with stabilization and degradation of their mRNA and to control their mRNA quality by binding with variety of RBPs.

RIP-Certified Antibody

Code No.	Product name (Gene name)	Clone Application	Isotype Species reactivity	Size	Function, localization, etc
RN033P	anti-TNRC6A (GW182, GW1)	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo,Rat	200 µL	TNRC6A/GW182 is related with translational inhibition via binding with RISC forming factors, and is one of the critical components of P-body/GW-body as well.
RN001P	anti-EIF4E	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo,Rat,Hm	200 µL	EIF4E, one of the translational initiation factors, binds to m^7G cap structure. Binding with inhibitory protein 4E-BP inhibits its binding to eIF4G1.
RN004P	anti-ELAVL1 (HuR)	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo,Rat,Hm	200 µL	ELAVL1/HuR is ubiquitously expressed and binds with both poly(A) and AU-rich element. It shuttles between nucleus and cytoplasm and may associate with transport of mRNA.
RN005P	anti-ELAVL2 (HuB)	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu	200 µL	ELAVL2/HuB is one of the members of Hu family proteins that include HuR, HuB, HuC and HuD. Specific expression in nerve system has been reported.
RN006P	anti-ELAVL3 (HuC)	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo,Rat	200 µL	ELAVL3/HuC is one of the members of Hu family proteins that include HuR, HuB, HuC and HuD. It stabilizes mRNA and regulates the efficiency of translation via binding with ARE.
RN045P	anti-SLBP (HBP)	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo,Rat,Hm	200 µL	SLBP binds to stem-loop structure in 3' end of histone pre-mRNA and is related with processing, transport, translation, degradation and cell cycle regulation.
RN011P	anti-PTBP1 (hnRNPI)	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu	200 µL	PTBP1 is related with alternative splicing and negatively regulates the splice site via binding with intronic cluster of pre-mRNA.
RN021P	anti-KHDRBS1 (p62, SAM68)	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo,Rat	200 µL	KHDRBS1, a DNA/RNA binding protein, affects inclusion of CD44 exon v5 via regulating alternative splicing. Some reports indicate it is related with stabilization and translational regulation of mRNA.
RN022P	anti-PABPC4 (PABP4, iPABP)	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo,Rat	200 µL	PABPC4, associated with mRNA stabilization and translational regulation, shares 79% of homology with PABPC. PABP4 stabilizes mRNA via binding with poly(A).
RN024P	anti-PCBP1 (HNRPE1)	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo,Rat	200 µL	PCBP1 stabilizes the target mRNAs such as those of α-globin, type I collagen, and tyrosine hydroxylase via binding with poly(rC) and polypyrimidine.
RN025P	anti-PCBP2 (HNRPE2)	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo,Rat	200 µL	PCBP2 binds to poly(rC) in the same way as PCBP1 and hnRPK, and has been reported to bind to poly(rU) as well. It is localized in stress granule and P-body.

Code No.	Product name (Gene name)	Clone Application	Isotype Species reactivity	Size	Function, localization, etc
RN026P	anti-PUM1	Polyclonal	rab Ig (aff.)	200 µL	PUM1, one of the members of PUF family, has a Pumilio homology domain as a RNA binding domain. It is related with acceleration of degradation via binding with 3'-UTR of target mRNA.
		WB,IPP,RIP	Hu,Mo		
RN027P	anti-PUM2	Polyclonal	rab Ig (aff.)	200 µL	PUM2 is one of the members of PUF family associated with regulation of development. PUM2 has a same sublocalization with PUM1 in variety of tissues.
		WB,IPP,RIP	Hu,Mo,Rat		
RN037P	anti-AUH	Polyclonal	rab Ig (aff.)	200 µL	AUH regulates the stability of mRNA via binding with AU-rich element of mRNA of early response gene such as IL-3, GM-CSF, c-fos and c-myc.
		WB,IPP,RIP	Hu,Mo,Rat,Hm		
RN020P	anti-ILF3	Polyclonal	rab Ig (aff.)	200 µL	ILF3 binds to dsRNA. It stabilizes mRNA via binding with AU-rich element of 3'-UTR of IL-2 mRNA, and is related with transport of mRNA from nucleus to cytoplasm.
	(NF90)	WB,IPP,RIP	Hu		
RN032P	anti-CIRBP	Polyclonal	rab Ig (aff.)	200 µL	CIRBP, related with cold-induced suppression of cell proliferation, serves as translational activator via increasing the translation level in the condition of mild cold stress.
	(CIRP)	WB,IPP,RIP	Hu,Mo,Rat		
RN003M	anti-EIF2C2	1B1-E2H5	mo IgG2a λ	200 µL	AGO2, a core component of RNA-induced silencing complex (RISC), takes a central role in RNAi pathway, and is sublocalized in P-body.
	(AGO2)	WB,IPP,RIP	Hu		
RN028P	anti-EIF2C1	Polyclonal	rab Ig (aff.)	200 µL	AGO1 takes a central role in RNAi pathway. It forms the RNA-induced silencing complex (RISC) that mediates gene silencing by RNA interference.
	(AGO1)	WB,IPP,RIP	Hu,Mo		

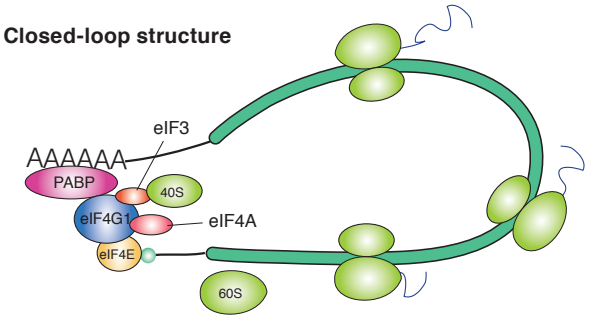
RBP Antibody

Code No.	Product name (Gene name)	Clone Application	Isotype Species reactivity	Size	Function, localization, etc
RN028PW	anti-EIF2C1	Polyclonal	rab Ig (aff.)	100 µL	AGO1 takes a central role in RNAi pathway. It forms the RNA-induced silencing complex (RISC) that mediates gene silencing by RNA interference.
	(AGO1)	WB,IPP	Hu,Mo		
RN003MW	anti-EIF2C2	1B1-E2H5	mo IgG2a λ	100 µL	AGO2, a core component of RNA-induced silencing complex (RISC), takes a central role in RNAi pathway, and is sublocalized in P-body.
	(AGO2)	WB,IPP	Hu		
RN029PW	anti-EIF2C2	Polyclonal	rab Ig (aff.)	100 µL	AGO2, a core component of RNA-induced silencing complex (RISC), takes a central role in RNAi pathway, and is sublocalized in P-body.
	(AGO2)	WB	Hu,Mo,Rat		
RN030PW	anti-DICER1	Polyclonal	rab Ig (aff.)	100 µL	DICER1, an endonuclease of the RNase III family, cleaves dsRNAs and pre-miRNAs into miRNA and siRNA duplex and loads them onto RISC.
	(DCR1)	WB,IPP	Hu		
RN002MW	anti-CUGBP1	3B1	mo IgG1 κ	100 µL	CUGBP1 binds to mRNAs of c-jun and TNFRSF1B through their GU-rich element and regulate their alternative splicing and degradation.
	(CELF1)	WB,IPP	Hu,Mo,Rat		
RN034PW	anti-CUGBP1	Polyclonal	rab Ig (aff.)	100 µL	It has been reported that CUGBP1 plays a role in the pathogenesis of the trinucleotide expansion disease, namely, myotonic dystrophy (DM1).
	(CELF1)	WB	Hu,Mo,Rat,Hm		
RN035PW	anti-CUGBP2	Polyclonal	rab Ig (aff.)	100 µL	CUGBP2 is not expressed only in muscle but is ubiquitous. It stabilizes COX2 mRNA via binding to 3'-UTR. It induces apoptosis in tumor cells via binding with 3'-UTR of Mcl-1 mRNA.
	(CELF2)	WB	Hu,Mo,Rat,Hm		
RN023PW	anti-PABPN1	Polyclonal	rab Ig (aff.)	100 µL	PABPN1, sublocalized in nucleus, strongly binds with poly(A) of primary mRNA. It is essential for polyadenylation and is associated with maturation of mRNA via regulation of the length of poly(A).
	(PABP2)	WB,IPP	Hu,Mo		
RN031PW	anti-ZFP36	Polyclonal	rab Ig (aff.)	100 µL	ZFP36 has a zinc finger structure and binds to ARE of mRNA. It recruits deadenylase complex and induces deadenylation of poly(A) tail via binding with ARE.
	(TTP)	WB	Hu		
RN036PW	anti-ACO1	Polyclonal	rab Ig (aff.)	100 µL	ACO1 has been reported to stabilize of transferrin receptor mRNA as well via binding with 3'-UTR of transferrin receptor mRNA.
	(IRP1, IREB1)	WB,IPP	Hu,Mo,Rat,Hm		

Species reactivity : Hu: Human, Mo: Mouse, Rat: Rat, Hm: Hamster Application : WB: Western blotting, IPP: Immunoprecipitation, RIP: RIP-assay

Regulation of translation

Initiation of efficient and optimal translation of mRNA depends on the formation of a closed-loop structure. Basically, it is formed by eIF4E, a cap-binding protein, and a poly(A) binding protein (PABP) through a scaffold protein, eIF4G1, as a linker. Many proteins, such as Hu proteins, a family of RBPs, are suggested to be involved in stabilization of this closed-loop structure.



RIP-Certified Antibody

Code No.	Product name (Gene name)	Clone Application	Isotype Species reactivity	Size	Function, localization, etc
RN033P	anti-TNRC6A (GW182, GW1)	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo,Rat	200 µL	TNRC6A/GW182 is related with translational inhibition via binding with RISC forming factors. It posesses RNA recognition motif and multiple AGO binding domains, and related with gene silencing.
RN001P	anti-EIF4E	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo,Rat,Hm	200 µL	EIF4E, one of the translational initiation factors, binds to m^7G cap structure. Binding with inhibitory protein 4E-BP inhibits its binding to eIF4G1.
RN002P	anti-EIF4G1	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu	200 µL	EIF4G1 functions as a scaffold protein necessary for cap-dependent translation.
RN003P	anti-EIF4G2	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo,Rat,Hm	200 µL	EIF4G2 is homologous with C-term of eIF4G1 and functions as translational inhibitor via forming an inactive complex with eIF4A and eIF3.
RN038P	anti-CPEB1	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu	200 µL	CPEB1 binds to CPE and regulates translation of mRNA via Maskin like protein. When phosphorylated by kinase such as Aurora, it facilitates translation of mRNA by interacting with CPSF.
RN004P	anti-ELAVL1 (HuR)	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo,Rat,Hm	200 µL	ELAVL1/HuR is ubiquitously expressed and binds with both poly(A) and AU-rich element. It shuttles between nucleus and cytoplasm and may associate with transport of mRNA.
RN005P	anti-ELAVL2 (HuB)	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu	200 µL	ELAVL2/HuB is one of the members of Hu family proteins that include HuR, HuB, HuC and HuD. It stabilizes mRNA and regulates the efficiency of translation via binding with ARE.
RN006P	anti-ELAVL3 (HuC)	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo,Rat	200 µL	ELAVL3/HuC is one of the members of Hu family proteins that include HuR, HuB, HuC and HuD. Specific expression in nerve system has been reported.
RN010P	anti-MSI1 (Musashi1)	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo,Rat	200 µL	MSI1 regulates the differentiation of neural stem cell via translational inhibition by binding with 3'-UTR of Numb mRNA.
RN001M	anti-IGF2BP1 (IMP1, ZBP1)	6H6 WB,IPP,RIP	mo IgG2a κ Hu,Mo	200 µL	Together with FMRP, IGF2BP1 binds to β-actin mRNA and transports it from nucleus to cytoplasm.
RN007P	anti-IGF2BP1 (IMP1, ZBP1)	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo	200 µL	IGF2BP1 binding inhibits the initiation of translation, whereas in the destination of cytoplasm, the IGF2BP1 is released from mRNA by phosphorylation to initiate the translation.
RN008P	anti-IGF2BP2 (IMP2)	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo,Rat	200 µL	IGF2BP2/IMP2 binds to UTR region of IGF2 leader 3 mRNA, which is related with growth and proliferation, and regulates their translation.
RN009P	anti-IGF2BP3 (IMP3)	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo	200 µL	IGF2BP3/IMP3 binds to 5'-UTR region of IGF2 leader 3 mRNA, which is related with growth and proliferation, and regulates their translation.
RN045P	anti-SLBP (HBP)	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo,Rat,Hm	200 µL	SLBP binds to stem-loop structure in 3' end of histone pre-mRNA and is related with processing, transport, translation, degradation and cell cycle regulation.
RN019P	anti-HNRNPK (HNRPK)	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo,Rat	200 µL	HNRPK is related with transport and translational regulation of mRNA. Phosphorylation of hnRNPK leads to cytoplasmic accumulation and is important for cell migration and metastasis.
RN014P	anti-TIA1	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo,Rat,Hm	200 µL	TIA1 shuttles between nucleus and cytoplasm. It is related with inducing apoptosis, translational regulation of TNF-α and COX-2 as well as alternative splicing of genes including Fas and FGFR2.
RN015P	anti-YBX1	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo,Rat,Hm	200 µL	YBX1 is one of the main components of cytoplasmic mRNP particles. It is related with regulation of gene expression at transcriptional/translational level via binding with DNA/RNA.

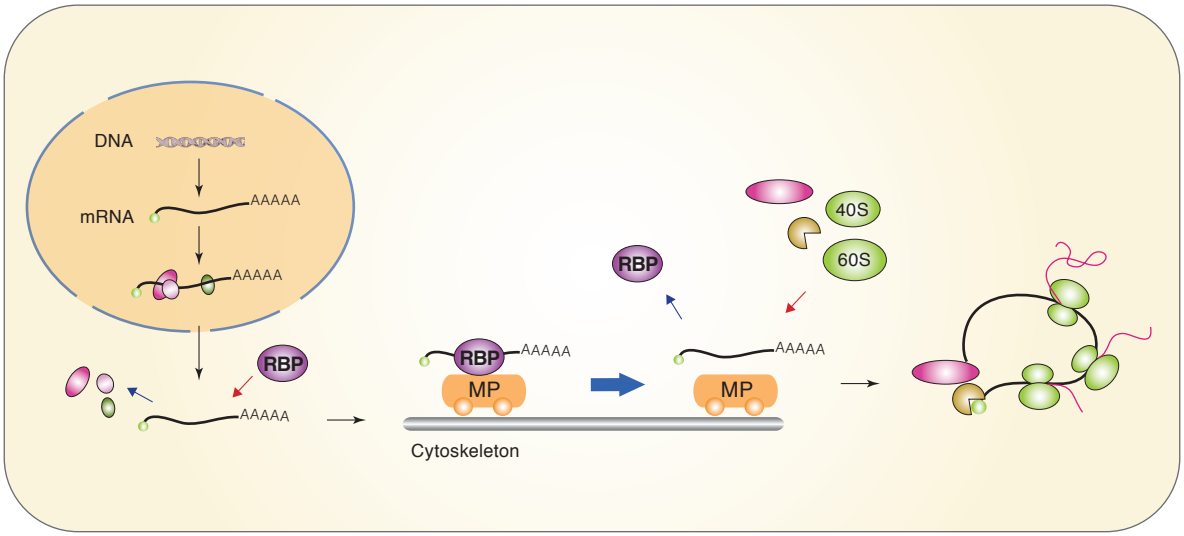
Code No.	Product name (Gene name)	Clone Application	Isotype Species reactivity	Size	Function, localization, etc
RN021P	anti-KHDRBS1	Polyclonal	rab Ig (aff.)	200 µL	KHDRBS1, a DNA/RNA binding protein, regulates splicing via binding CD44 exon v5. Some reports indicate it is related with stabilization and translational regulation of mRNA.
	(p62, SAM68)	WB,IPP,RIP	Hu,Mo,Rat		
RN016P	anti-FMR1	Polyclonal	rab Ig (aff.)	200 µL	FMR1 is necessary for morphogenesis of nerve system and strongly binds with poly(G) sequence. The expansion of CGG repeats within the FMR gene is found in Fragile X syndrome patients.
	(FMRP)	WB,IPP,RIP	Hu,Mo,Rat		
RN017P	anti-FXR1	Polyclonal	rab Ig (aff.)	200 µL	FXR1, known as a paralogue of FMR1,shuttles between nucleus and cytoplasm, and binds with 60S subunit of polysome. It may relate with AGO protein as well.
		WB,IPP,RIP	Hu,Mo,Rat		
RN018P	anti-FXR2	Polyclonal	rab Ig (aff.)	200 µL	FXR2, functionally related with FMR1 and FXR, shares high homology with FXR1. They include binding with RNA and polysome, and shuttling between nucleus and cytoplasm.
		WB,IPP,RIP	Hu,Mo,Rat		
RN024P	anti-PCBP1	Polyclonal	rab Ig (aff.)	200 µL	PCBP1 is one of the isoform of PCBP that shuttles between nucleus and cytoplasm. It functions as translational co-activator via binding with stem-loop structure of IRES of poliovirus.
	(HNRPE1)	WB,IPP,RIP	Hu,Mo,Rat		
RN025P	anti-PCBP2	Polyclonal	rab Ig (aff.)	200 µL	PCBP2 binds to poly(rC) in the same way as PCBP1 and hnRPK, and has been reported to bind to poly(rU) as well. It is localized in stress granule and P-body.
	(HNRPE2)	WB,IPP,RIP	Hu,Mo,Rat		
RN026P	anti-PUM1	Polyclonal	rab Ig (aff.)	200 µL	PUM1, one of the members of PUF family, has a Pumilio homology domain as a RNA binding domain. It is related with translational inhibition via binding with 3'-UTR of target mRNA.
		WB,IPP,RIP	Hu,Mo		
RN027P	anti-PUM2	Polyclonal	rab Ig (aff.)	200 µL	PUM2 is one of the members of PUF family associated with regulation of development. Some reports indicate being related with differentiation of germ cell.
		WB,IPP,RIP	Hu,Mo,Rat		
RN020P	anti-ILF3	Polyclonal	rab Ig (aff.)	200 µL	ILF3 binds to dsRNA. It stabilizes mRNA via binding with AU-rich element of 3'-UTR of IL-2 mRNA, and is related with transport of mRNA from nucleus to cytoplasm.
	(NF90)	WB,IPP,RIP	Hu		
RN032P	anti-CIRBP	Polyclonal	rab Ig (aff.)	200 µL	CIRBP, related with cold-induced suppression of cell proliferation, serves as translational activator via increasing the translation level in the condition of mild cold stress.
	(CIRP)	WB,IPP,RIP	Hu,Mo,Rat		
RN003M	anti-EIF2C2	1B1-E2H5	mo IgG2a λ	200 µL	AGO2, a core component of RNA-induced silencing complex (RISC), takes a central role in RNAi pathway, and is sublocalized in P-body.
	(AGO2)	WB,IPP,RIP	Hu		
RN028P	anti-EIF2C1	Polyclonal	rab Ig (aff.)	200 µL	AGO1 takes a central role in RNAi pathway. It forms the RNA-induced silencing complex (RISC) that mediates gene silencing by RNA interference.
	(AGO1)	WB,IPP,RIP	Hu,Mo		

RBP Antibody

Code No.	Product name (Gene name)	Clone Application	Isotype Species reactivity	Size	Function, localization, etc
RN028PW	anti-EIF2C1	Polyclonal	rab Ig (aff.)	100 µL	AGO1 takes a central role in RNAi pathway. It forms the RNA-induced silencing complex (RISC) that mediates gene silencing by RNA interference.
	(AGO1)	WB,IPP	Hu,Mo		
RN003MW	anti-EIF2C2	1B1-E2H5	mo IgG2a λ	100 µL	AGO2, a core component of RNA-induced silencing complex (RISC), takes a central role in RNAi pathway, and is sublocalized in P-body.
	(AGO2)	WB,IPP	Hu		
RN029PW	anti-EIF2C2	Polyclonal	rab Ig (aff.)	100 µL	AGO2, a core component of RNA-induced silencing complex (RISC), takes a central role in RNAi pathway, and is sublocalized in P-body.
	(AGO2)	WB	Hu,Mo,Rat		
RN030PW	anti-DICER1	Polyclonal	rab Ig (aff.)	100 µL	DICER1, an endonuclease of the RNase III family, cleaves dsRNAs and pre-miRNAs into miRNA and siRNA duplex and loads them onto RISC.
	(DCR1)	WB,IPP	Hu		
RN039PW	anti-CPEB2	Polyclonal	rab Ig (aff.)	100 µL	CPEB2 regulates the polyadenylation and translation of mRNA. It binds to HIF-1α mRNA induced by low oxygen stimulation.
		WB,IPP	Hu,Rat		
RN040PW	anti-CPEB4	Polyclonal	rab Ig (aff.)	100 µL	Together with CPEB1, CPEB4 regulates mitotic cell-cycle progression, mRNA polyadenylation and translation.
		WB,IPP	Hu		
RN036PW	anti-ACO1	Polyclonal	rab Ig (aff.)	100 µL	ACO1/IRP1 inhibits the translation of ferritin mRNA via binding with 5'-UTR of ferritin mRNA in the low ferrous ion condition.
	(IRP1, IREB1)	WB,IPP	Hu,Mo,Rat,Hm		

Species reactivity : Hu: Human, Mo: Mouse, Rat: Rat, Hm: Hamster Application : WB: Western blotting, IPP: Immunoprecipitation, RIP: RIP-assay

Localization

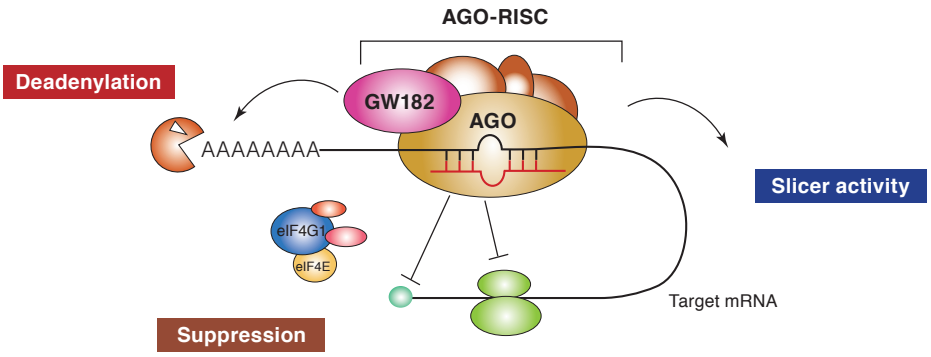


Following the RNA export from nucleus to cytoplasm, mRNAs are transported along cytoskeleton to peripheral end with the aid of motor protein (MP) and RBPs that regulate localization of mRNAs. RBPs protect mRNAs from degradation by binding to their target mRNAs during the localization. In this manner, the mRNA localization in cytoplasm is regulated to enable the efficient translation, which occurs at the “right place” on the “right time”. Recent studies have shown that RBPs play an important role in regulation of mRNA translation with spacio-temporal manner.

RIP-Certified Antibody

Code No.	Product name (Gene name)	Clone Application	Isotype Species reactivity	Size	Function, localization, etc
RN001M	anti-IGF2BP1 (IMP1, ZBP1)	6H6 WB,IPP,RIP	mo IgG2a κ Hu,Mo	200 µL	Together with FMRP, IGF2BP1 binds to β-actin mRNA and transports it from nucleus to cytoplasm.
RN007P	anti-IGF2BP1 (IMP1, ZBP1)	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo	200 µL	IGF2BP1 binding inhibits the initiation of translation, whereas in the destination of cytoplasm, the IGF2BP1 is released from mRNA by phosphorylation to initiate the translation.
RN008P	anti-IGF2BP2 (IMP2)	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo,Rat	200 µL	IGF2BP2/IMP2 binds to UTR region of IGF2 leader 3 mRNA, which is related with growth and proliferation, and regulates their translation.
RN009P	anti-IGF2BP3 (IMP3)	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu,Mo	200 µL	IGF2BP3/IMP3 binds to 5'-UTR region of IGF2 leader 3 mRNA, which is related with growth and proliferation, and regulates their translation.
RN012P	anti-STAU1	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu	200 µL	STAU1 forms RNA granule in neuron. It is related with transport and sublocalization of mRNA. It is also reported to be related with NMD and binds to 40S and 60S of the ribosomal subunit.
RN013P	anti-STAU2	Polyclonal WB,IPP,RIP	rab Ig (aff.) Hu	200 µL	STAU2 is highly homologous to STAU1. Different sublocalization with STAU1 in neuron indicates that STAU2 may be related with forming of different RNA granule.

RNAi pathway (miRNA/siRNA/piRNA)



RNA interference (RNAi) is a mechanism that controls the gene expressions in a sequence specific manner. Small ncRNAs, including miRNAs, siRNAs as well as piRNAs, function as the guide molecules that incorporated into RNA-induced silencing complex (RISC). They control the translation and degradation of their target mRNAs that have the complementary sequences with their guide strands. RNAi has recently been shown to play an important role in a variety of biological phenomena including the dynamics of early development, morphogenesis, cell growth and tumorigenesis.

Small ncRNA does not function directly by itself. It functions only when it is incorporated into Argonaute (AGO) protein, a key component of RISC. It functions as a guide to recognize the target mRNA. The translational inhibition and degradation of the target mRNA are due to the activities of the RISC components including AGO and GW182. Recently, post-transcriptional regulation through these small ncRNAs is attracting the attention of researchers.

RIP-Certified Antibody

Code No.	Product name (Gene name)	Clone Application	Isotype Species reactivity	Size	Function, localization, etc
RN003M	anti-EIF2C2	1B1-E2H5	mo IgG2a λ	200 µL	AGO2, a core component of RNA-induced silencing complex (RISC), takes a central role in RNAi pathway, and is sublocalized in P-body.
	(AGO2)	WB,IPP,RIP	Hu		
RN033P	anti-TNRC6A	Polyclonal	rab Ig (aff.)	200 µL	TNRC6A/GW182 is related with translational inhibition via binding with RISC forming factors, and is one of the critical components of P-body/GW-body as well.
	(GW182, GW1)	WB,IPP,RIP	Hu,Mo,Rat		
RN028P	anti-EIF2C1	Polyclonal	rab Ig (aff.)	200 µL	AGO1 takes a central role in RNAi pathway. It forms the RNA-induced silencing complex (RISC) that mediates gene silencing by RNA interference.
	(AGO1)	WB,IPP,RIP	Hu,Mo		

RBP Antibody

Code No.	Product name (Gene name)	Clone Application	Isotype Species reactivity	Size	Function, localization, etc
RN028PW	anti-EIF2C1	Polyclonal	rab Ig (aff.)	100 µL	AGO1 takes a central role in RNAi pathway. It forms the RNA-induced silencing complex (RISC) that mediates gene silencing by RNA interference.
	(AGO1)	WB,IPP	Hu,Mo		
RN003MW	anti-EIF2C2	1B1-E2H5	mo IgG2a λ	100 µL	AGO2, a core component of RNA-induced silencing complex (RISC), takes a central role in RNAi pathway, and is sublocalized in P-body.
	(AGO2)	WB,IPP	Hu		
RN029PW	anti-EIF2C2	Polyclonal	rab Ig (aff.)	100 µL	AGO2, a core component of RNA-induced silencing complex (RISC), takes a central role in RNAi pathway, and is sublocalized in P-body.
	(AGO2)	WB	Hu,Mo,Rat		
RN030PW	anti-DICER1	Polyclonal	rab Ig (aff.)	100 µL	DICER1, an endonuclease of the RNase III family, cleaves dsRNAs and pre-miRNAs into miRNA and siRNA duplex and loads them onto RISC.
	(DCR1)	WB,IPP	Hu		

Species reactivity : Hu: Human, Mo: Mouse, Rat: Rat, Hm: Hamster Application : WB: Western blotting, IPP: Immunoprecipitation, RIP: RIP-assay

RiboCluster Profiler™

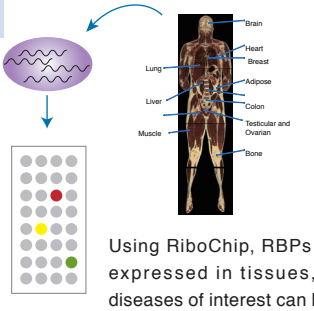
The cyclic discovery of RBPs and their targets is described by the Ribonomic Discovery Cycle. RiboCluster Plofiler™ offers multiple entering points for customers who have gene of interest, RBP of interest or tissue and disease of interest. Starting at the top and moving clockwise: When the customers are interested in specific disease or tissue, he/she can identify disease specific or tissue specific RBPs by using RiboChip. Following the RiboChip, RNP Immunoprecipitation (RIP) will be performed by RIP-Certified Antibody to isolate mRNAs from RNP complex. MBL offers **RIP-Assay Kit (RN1001)**, **RIP-Assay Kit *for microRNA* (RN1005)** and RIP-Certified Antibodies for RNP Immunoprecipitation. Isolated mRNAs, miRNAs and ncRNAs are analyzed by microarray, sequencing or RT-PCR. The RIP-Assay data provides customers with valuable information that cannot be obtained by a conventional gene expression analysis.

When the customers are interested in specific RNA, he/she can identify specific RBPs that bind to the RNA of interest to understand protein components consist the regulatory machinery of mRNA expression. MBL offers an immunopurification tool, **RiboTrap Kit (RN1011/RN1012)**, for identification of RBPs that bind to the RNA of interest. Following the RiboTrap, RIP-Chip or RIP-Seq can be performed to understand regulatory components in post-transcriptional regulation of gene expression or biosynthesis of small RNA and ncRNA.

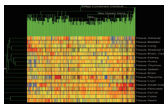
Additional products may become available. Please visit our website (https://ruo.mbl.co.jp/je/rip-assay/) to find out the latest product information.

RiboChip

Enables customers to profile tissue and/or disease specific RBP expression



Using RiboChip, RBPs specifically expressed in tissues, cells, and diseases of interest can be identified. Then the one of the RBPs enters into the Ribonomic Discovery Cycle.



RiboTrap

Enables customers to identify endogenous RBPs that interact with mRNA of interest

RiboTrap Kit

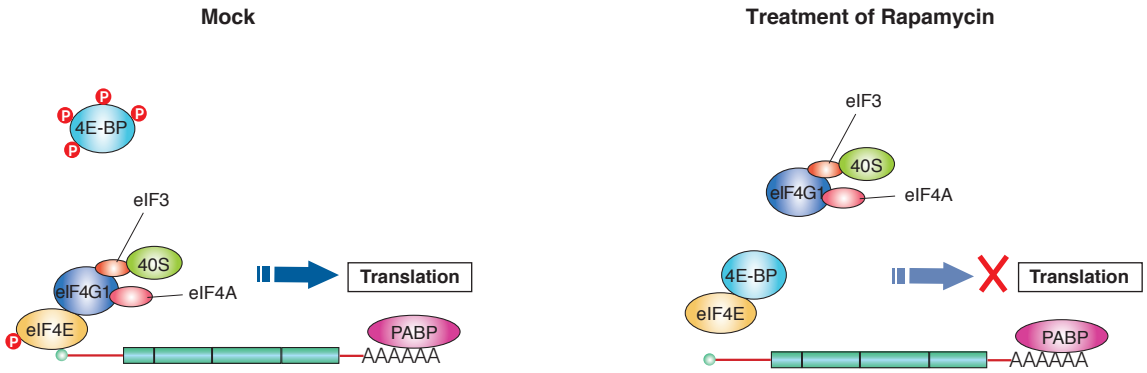
RIP-Chip

Enables customers to isolate mRNP complex and profile RNAs associated with the target RBP

RIP-Assay Kit
RIP-Assay Kit *for microRNA*
RIP-Certified Antibody

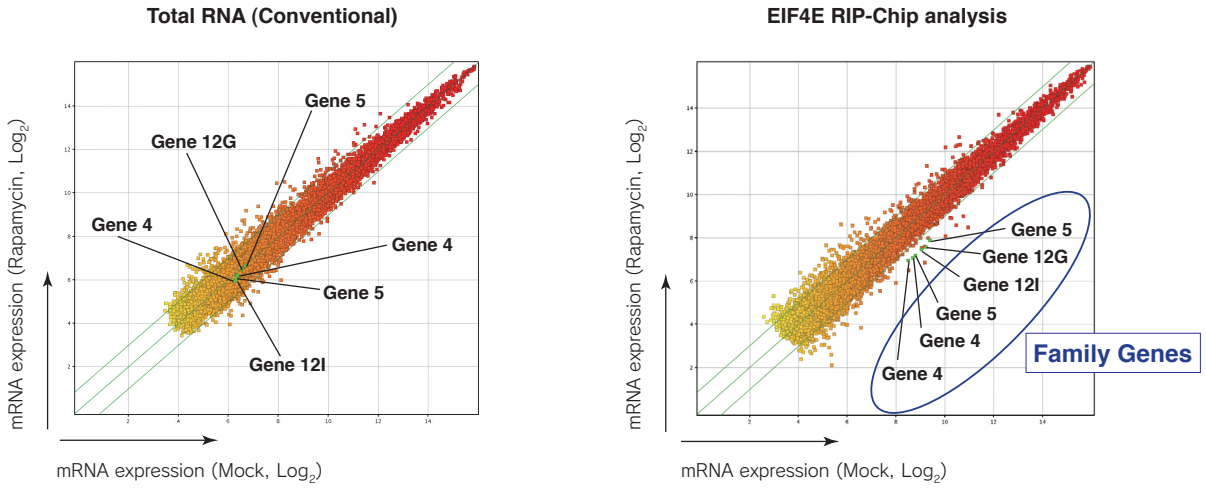
Reference
Dale L. Beach and Jack D. Keene,
Methods Mol. Biol., 419: 69-91. (2008)

Analysis of mRNAs of which binding status with eIF4E are changed by the treatment of Rapamycin.



Unlike conventional gene expression analysis based on the measurement of mRNAs, RIP-Chip is a tool to compare the binding status of RNAs with RBPs that have specific functions. For example, when a 5'-cap binding protein, eIF4E, is used as a target RBP for RIP-Chip analysis, it is possible to detect the changes of the population of target mRNAs affected by the treatment with Rapamycin (generic name: Sirolimus). The cap-binding complex eIF4F is involved in ribosome recruitment during the initiation phase of translation, thus the changes of the binding statuses with its target mRNAs are supposed to greatly affect the translational efficacy of its target mRNAs. Rapamycin is known to be capable of changing this binding status, thus used in clinical treatment as an immunosuppressant and anti-cancer agent.

Results



Expression profile of certain genes (Gene 4, 5, 12I, 12G) did not change in Rapamycin treated cells and in Mock sample.

Effect of Rapamycin at post-transcriptional level cannot be detected by conventional gene expression analysis.

Certain genes showed down-regulated expression profile in Rapamycin treated cells.

Effect of Rapamycin at post-transcriptional level can be detected by EIF4E RIP-Chip analysis.

RIP-Chip makes it possible to extensively analyze the changes occurred at translational level, which is impossible to be observed by using conventional method.

The expression levels of the mRNAs, indicated green spots, have not changed by treatment of Rapamycin, however, the cap-binding ratio is decreased. That is to say, drug treatment decreases the translational efficiency of these mRNAs, and the expression of the proteins is regulated at translational level rather than at transcriptional level.

RBP is known to regulate the mRNAs that are functionally related via cluster. RIP-Chip analysis using anti-EIF4E antibody (right graph) showed that Gene 4, 5, 12I and 12G were down-regulated. We found that those mRNAs were functionally related (data not shown).

RIP-Assay Kit

☞ Highly optimized, ready-to-use kit for RIP-Assay.

☞ Eco-friendly, no-phenol containing, proprietary buffers for RNA isolation.

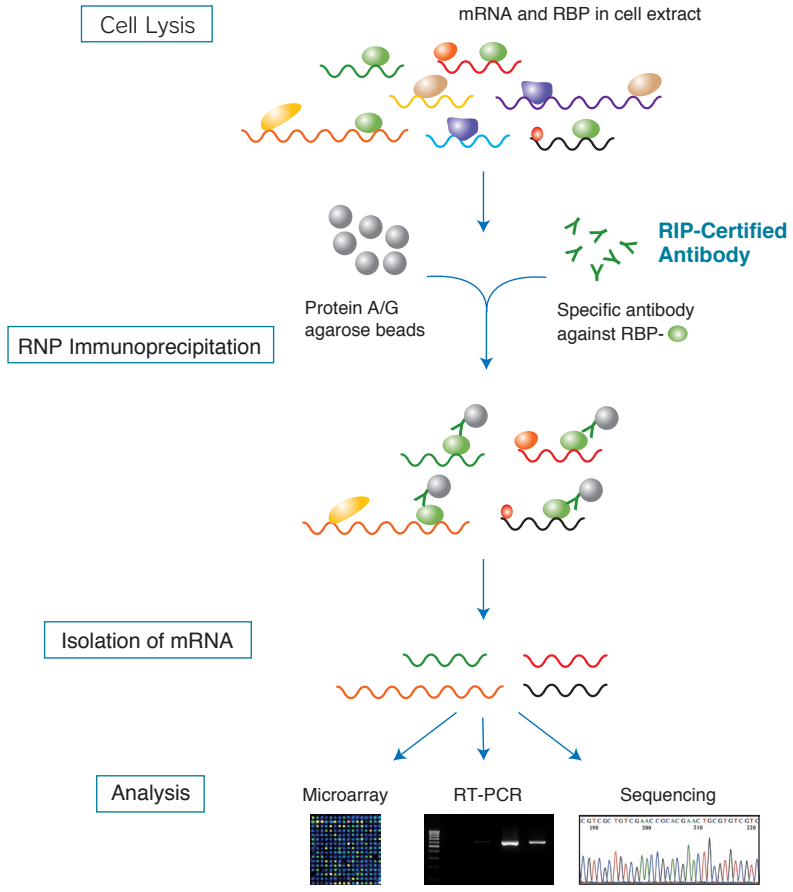
☞ High quality, high yield RNAs work for the all kind of post-RIP-Assay.

MBL has developed and marketed the RIP-Assay Kit, which enables customers to immunoprecipitate the mRNA-RBP complexes with RBP specific antibodies. The RIP-Certified Antibodies against a large variety of RBPs are also available from MBL.

RIP-Chip works on the same principle as the widely used ChIP-Chip. It immunoprecipitates the ribonucleoprotein (RNP) from the cell extracts using an antibody raised against the RBP of interest. This simple procedure is then followed by microarray analysis. While microarrays determine the sequences of the RNA targets by hybridization, direct sequencing approaches (RIP-Seq) can also be used to reveal RNA targets of RBPs.

RIP-Chip or RIP-Seq data provides insights into new cellular pathway components leading to potential therapeutic targets and can also provide informations regarding the effects of drugs on post-transcriptional processes. This technology can be applied to essentially any cellular system or animal model.

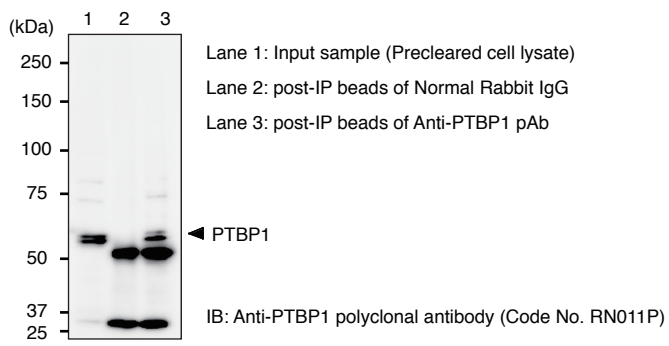
Principle of RIP-Assay



Example of RIP-Assay results

Cells:	Jurkat
Cell number:	6×10^6 cells/sample
Antibodies:	Normal Rabbit IgG
	Anti-PTBP1 pAb (Code No. RN011P)
Amount used:	15 µg

1. The binding of RNP with the beads was confirmed by WB after immunoprecipitation.



Lane 1: Input sample (Precleared cell lysate)

Lane 2: post-IP beads of Normal Rabbit IgG

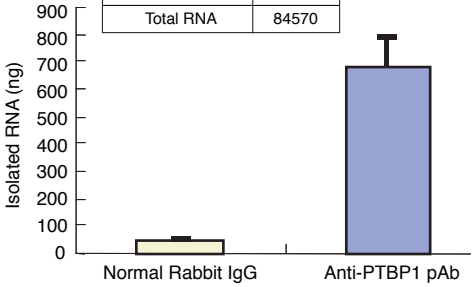
Lane 3: post-IP beads of Anti-PTBP1 pAb

IB: Anti-PTBP1 polyclonal antibody (Code No. RN011P)

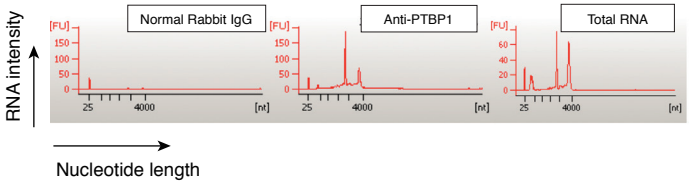
2. The amount of isolated RNAs was measured.

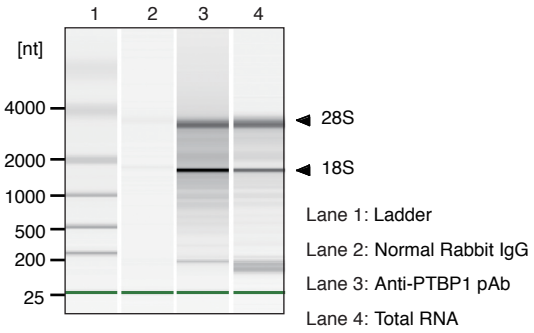
Average quantity of isolated RNA (n=2)

Antibody	RNA (ng)
Normal Rabbit IgG	51
Anti-PTBP1 pAb	678
Total RNA	84570

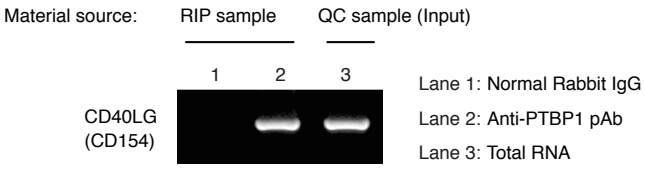


3. Isolated RNAs were analyzed by Bioanalyzer.





4. Isolated RNAs were identified by RT-PCR.



After the isolation of RNAs that bound with PTBP1 in Jurkat cells by using RIP-Assay Kit, the RNAs were identified by RT-PCR. CD40LG was detected at a significant level in the sample precipitated by anti-PTBP1 antibody rather than by control IgG. CD40LG has been reported to be target of PTBP1. Thus this data indicated the ability of RIP-Assay Kit for profiling the target mRNAs of RNP complex.

Kit components

Reagent	Size
Lysis Buffer	26 mL
Wash Buffer	35 mL × 2 bottles
Normal Rabbit IgG	200 µL
High-Salt Solution	6 mL
Solution I	260 µL
Solution II	10 mL
Solution III	7 mL
Solution IV	55 µL

Code No.	Product name	Size
RN1001	RIP-Assay Kit	10 assays

Materials required but not provided

- Protease inhibitor
- RNase inhibitor
- Protein A or Protein G agarose beads
- DTT

RIP-Assay Kit *for microRNA*

☞ It is possible to isolate miRNA and mRNA in one tube or in two (2) tubes separately.

☞ miRNA and mRNA can be isolated by antibodies against either RISC components or other RBPs.

☞ Eco-friendly, high efficient reagents are optimized for extraction of miRNAs/mRNAs work for all kinds of application.

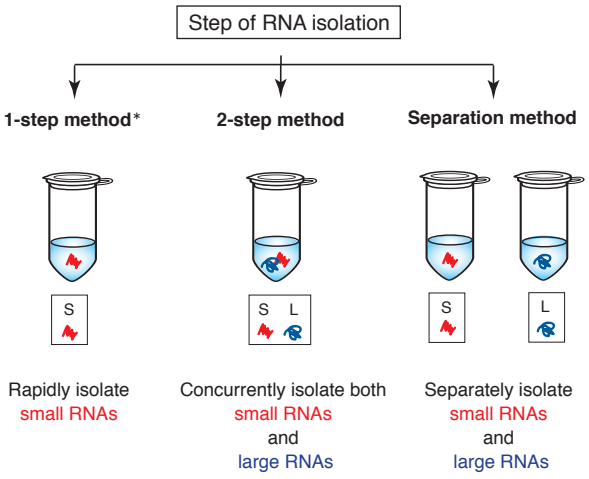
RIP-Assay Kit *for microRNA* is an optimized kit to isolate functionally related mRNAs and miRNAs. The kit provides three (3) different RNA isolation protocols on customer's demand.

. To rapidly isolate small RNAs
(1-step method)

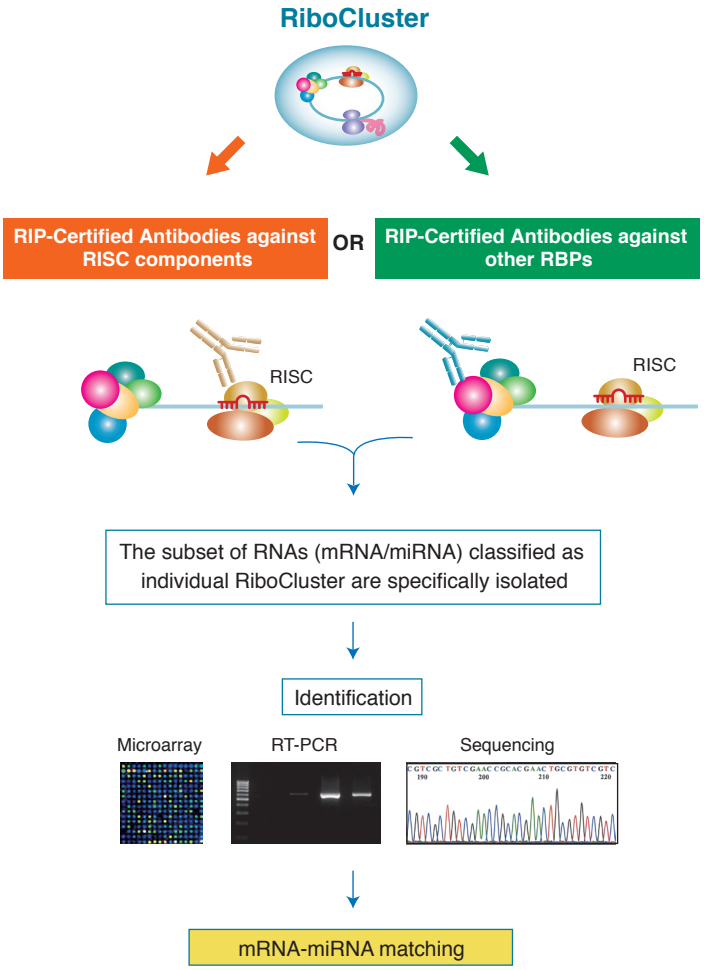
. To simultaneously isolate both small RNAs and large RNAs
(2-step method)

. To separately isolate small RNAs and large RNAs
(separation method)

Any one of these isolation methods recovers miRNA more efficiently than conventional phenol extraction method. This kit is useful to identify the disease or function related miRNAs as well as to extensively analyze their target mRNAs.



*This is not suitable for isolating large RNAs because the recovery for large RNAs is inefficient compared with the other 2 methods.



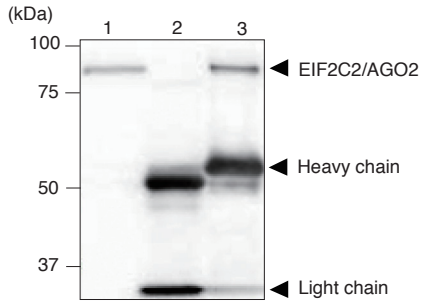
Example of RIP-Assay results 1

RISC components

RIP-Assay was performed by using antibody against AGO2 which takes a central role of RISC.

Cells:	Jurkat
Cell number:	1×10^7 cells/sample
Antibodies:	Mouse IgG2a λ (Code No. M076-3)
	Anti-AGO2 mAb (Code No. RN003M)
Amount used:	15 µg
RNA isolation:	Separation method

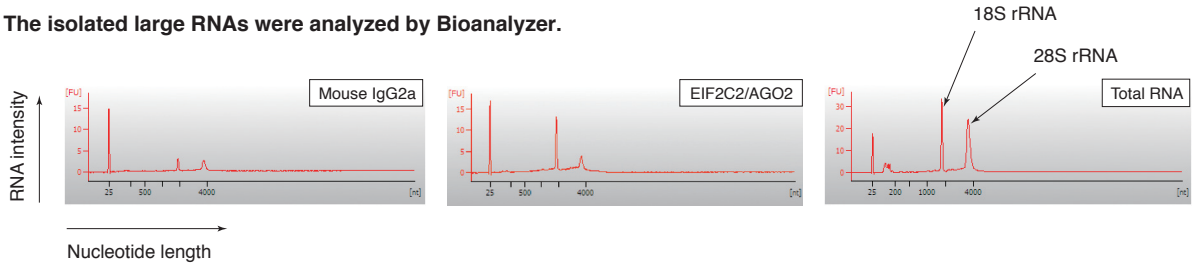
1. The binding of AGO2 with the beads was confirmed by WB after immunoprecipitation.



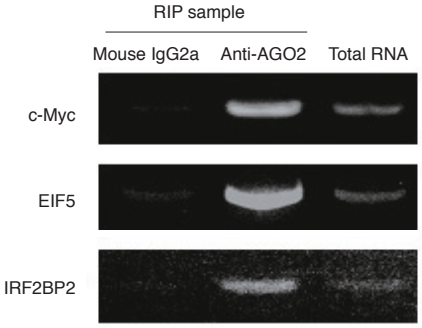
Lane 1: Input sample (Jurkat cell lysate)
Lane 2: post-IP beads of Mouse IgG2a
Lane 3: post-IP beads of Anti-EIF2C2/AGO2 mAb

IB: Anti-EIF2C2/AGO2 monoclonal antibody (Code No. RN003M)

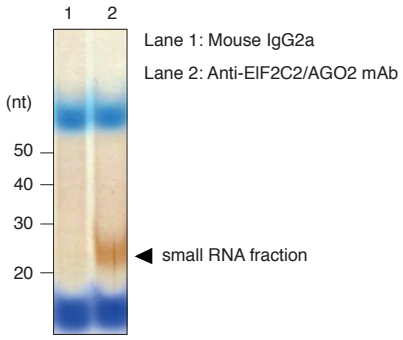
2. The isolated large RNAs were analyzed by Bioanalyzer.



3. The isolated large RNAs were analyzed by RT-PCR.

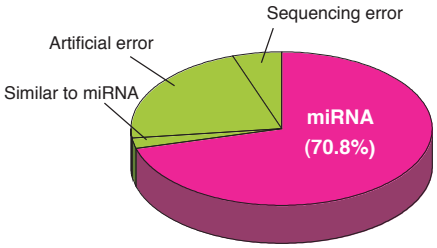


4. The isolated small RNAs were confirmed by silver staining.



Lane 1: Mouse IgG2a
Lane 2: Anti-EIF2C2/AGO2 mAb

5. Purified small RNAs were analyzed by sequencing.



miRNA:	coverage>90%, identity>90%
Similar to miRNA:	coverage>80%, identity>80%
Artificial error:	The error arising from cloning process
Sequencing error:	The error arising from sequencing process

Compared with the large RNA fraction prepared from post-IP beads of isotypic control, mRNAs of c-Myc, EIF5 and IRF2BP2 were highly enriched in the anti-AGO2 post-IP beads (3). Additionally, a variety of miRNAs were significantly enriched in the small RNA fraction of anti-AGO2 post-IP beads (4). Sequencing analysis showed that 70.8% of small RNAs were miRNAs (5). Excluding the artificial error and sequencing error, 90% of isolated small RNAs were miRNAs.

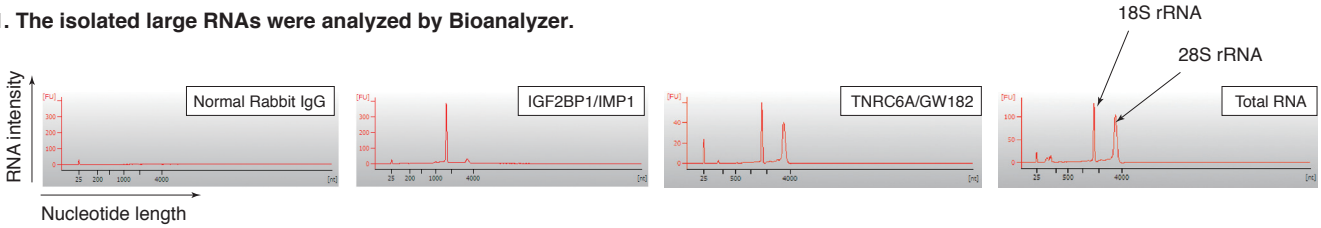
Example of RIP-Assay results 2

RISC components **Other RBPs**

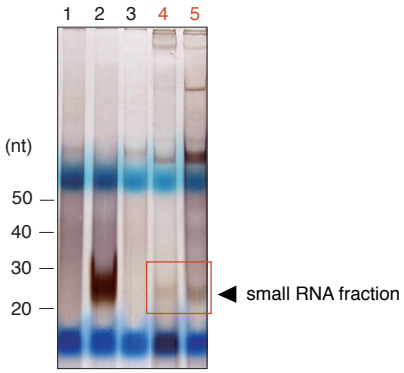
RIP-Assay was performed by using antibodies against IGF2BP1/IMP1 which is not a RISC component and TNRC6A/GW182 which is a RISC component.

Cells:	K562
Cell number:	1×10^7 cells/sample
Antibodies:	Mouse IgG2a λ (Code No. M076-3), Anti-AGO2 mAb (Code No. RN003M)
	Normal Rabbit IgG, Anti-IGF2BP1/IMP1 pAb (Code No. RN007P), Anti-TNRC6A/GW182 pAb (Code No. RN033P)
Amount used:	Mouse IgG2a λ, Anti-AGO2 mAb: 15 µg, Other antibodies: 25 µg
RNA isolation:	Separation method

1. The isolated large RNAs were analyzed by Bioanalyzer.

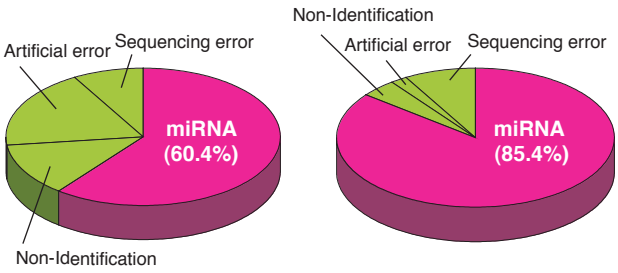


2. The isolated small RNAs were confirmed by silver staining.



Lane 1: Mouse IgG2a
Lane 2: Anti-EIF2C2/AGO2 mAb
Lane 3: Normal Rabbit IgG
Lane 4: Anti-IGF2BP1/IMP1 pAb
Lane 5: Anti-TNRC6A/GW182 pAb

3. Purified small RNAs were analyzed by sequencing.



IGF2BP1/IMP1 TNRC6A/GW182

miRNA:	(coverage>90%, identity>90%)
Non-Identification:	Non-Identification, may be novel miRNA sequencing
Artificial error:	The error arising from cloning process
Sequencing error:	The error arising from sequencing process

Compared with anti-AGO2 post-IP beads, less miRNAs were detected in the small RNA fractions of anti-IGF2BP1/IMP1, which is not a RISC component, and anti-TNRC6A/GW182, which is a RISC component (2). Sequencing analysis showed that the miRNAs identified in small RNA fractions obtained from anti-IMP1 post-IP beads and anti-GW182 post-IP beads were 60.4% and 85.4%, respectively (3).

Kit components

Reagent	Size
Immunoprecipitation reagents	
mi-Lysis Buffer	26 mL × 1 bottle
mi-Wash Buffer	35 mL × 2 bottles
Normal Rabbit IgG	0.33 mL × 1 vial
High-Salt Solution	6 mL × 1 vial
Reagents for RNA isolation	
mi-Solution I	0.26 mL × 1 vial
mi-Solution II	6 mL × 1 vial
mi-Solution III	4 mL × 1 vial
mi-Solution IV	0.2 mL × 1 vial
Gel extraction reagents	
Gel Extraction Buffer	25 mL × 1 vial
3 M NaOAc	1 mL × 1 vial
miSPIKE™	100 pmoles × 1 vial

Code No.	Product name	Size
RN1005	RIP-Assay Kit *for microRNA*	10 assays

RiboTrap Kit

☞ Immunoaffinity method to explore the RNA-protein and RNA-RNA interactions by using RNA of interest as a bait.

☞ No denaturing agent is used for elution, making it possible to work for a wide range of experimental purpose.

☞ Nucleus and cytoplasmic fraction can be extracted separately, making it possible to analyze the RNP components of both fractions.

The RiboTrap Kit is used to isolate RBPs and other proteins that are associated with mRNA, ribosomal RNA (rRNA), transfer RNA (tRNA), viral RNA, miRNA or any other RNA of interest from either the cytoplasmic or nuclear extract of cultured mammalian cells.

The RNA of interest is modified with 5-bromo-UTP (BrUTP) by *in vitro* transcription, followed by incubation with a cell lysate (cytoplasmic extract or nuclear extract) to form the assembled RNA-RBP complexes. The BrU-labeled RNA-RBP complexes are then immunoaffinity-purified by anti-BrdU monoclonal antibody (mAb), which cross-reacts with BrUTP. RBPs associated with the BrU-labeled RNA can be identified by immunoblotting or mass spectroscopy.

Three (3) different wash buffers provided in the RiboTrap Kit allow for analysis of both weakly and tightly bound RBPs.

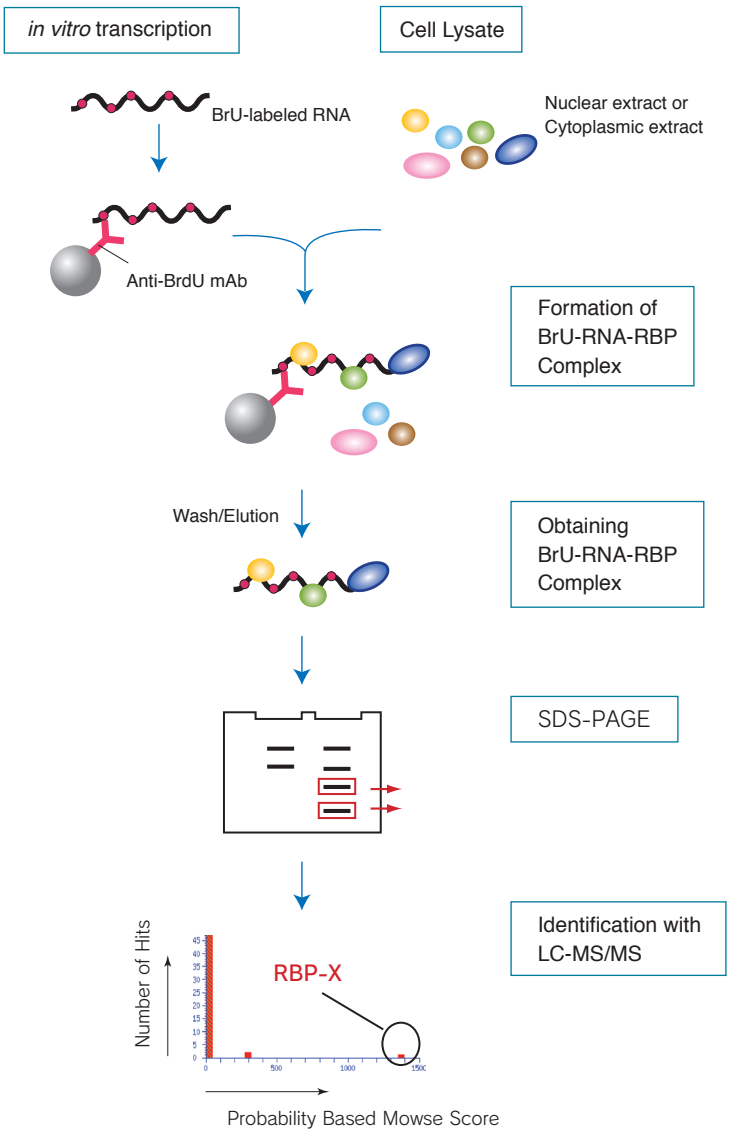
· Wash Buffer I : mild condition (primary screening)

· Wash Buffer II : stringent condition (high-ionic strength)

· Wash Buffer III : stringent condition (strong detergent)

Antisense RNA (corresponding to complementary RNA of interest) or truncated RNA of interest is used to exclude nonspecific binding proteins. Elution buffer composed of the optimal concentration of BrdU allows specific recovery of BrU-RNA/protein complexes. RBPs isolated with native conformations can be used in several downstream applications.

Principle of RiboTrap Kit



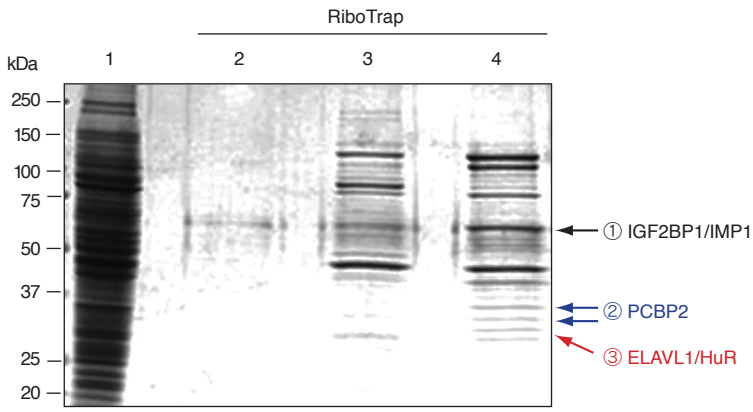
Comparison of three different wash buffers

	Wash Buffer I (Basic buffer)	Wash Buffer II	Wash Buffer III
Ionic strength	Low	High	Low
Detergent strength	Mild	Mild	Strong
Wash conditions	Mild	Stringent	Stringent
Retained tightly bound RBPs	○	○	○
Retained weakly bound RBPs	○	×	×
Contamination of nonspecifically bound RBPs	Likely present	Negligible	Negligible

Example of RiboTrap results

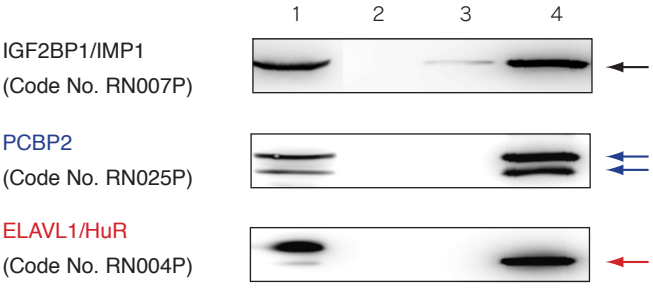
Cells:	HEK293T
Cell number:	8×10^7 cells
Cell lysate:	Cytoplasmic fraction
Bait RNA:	p21 coding sequence (0.5 kb), p21 3'-UTR (1.5 kb)
Wash Buffer:	Wash Buffer I

1. The isolated proteins were resolved by SDS-PAGE.



Lane 1: Input (Cytoplasmic extract)
Lane 2: Beads alone
Lane 3: p21 coding sequence
Lane 4: p21 3′- UTR

2. Endogeneous proteins bound to bait RNA were analyzed by Western blotting.



BrU-labeled 3'-UTR of p21 mRNA was incubated with cytoplasmic fraction of HEK293T cells to form mRNA-protein complex followed by immunoaffinity-purification using anti-BrdU antibody.
Endogenous proteins bound to bait RNA were identified by LC-MS/MS, and these binding statuses were confirmed by Western blotting using antibodies against each protein.

Kit components

Reagent	Size
[RN1012]	
Reagents for cell lysis (Store at 2-8°C)	
CE Buffer	15 mL × 1 bottle
CE Wash Buffer	13 mL × 3 bottles
NE Buffer	6 mL × 1 bottle
Dilution Buffer	9 mL × 1 bottle
Detergent Solution	0.75 mL × 1 vial
High-Salt Solution	0.45 mL × 1 vial
Wash reagents (Store at 2-8°C)	
Wash Buffer I	48 mL × 1 bottle
Wash Buffer II	48 mL × 1 bottle
Wash Buffer III	48 mL × 1 bottle
Beads Wash Buffer	38 mL × 1 bottle

Reagent	Size
[RN1011]	
RiboTrap reagents (Store at -20°C)	
Anti-BrdU mAb	0.5 mL × 1 vial
BrdU/DMSO	0.05 mL × 1 vial
5-Bromo-UTP (50 mM)	0.018 mL × 1 vial
Column (For Elution) ‡	10 columns

‡ Column can be stored at 2-8°C or -20°C or room temperature.

Code No.	Product name	Size
RN1011 RN1012*	RiboTrap Kit	10 assays

*RN1012 and RN1011 are sold as a set.
RN1012 and RN1011 should be stored at different temperature.
RN1011: -20°C RN1012: 2-8°C

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