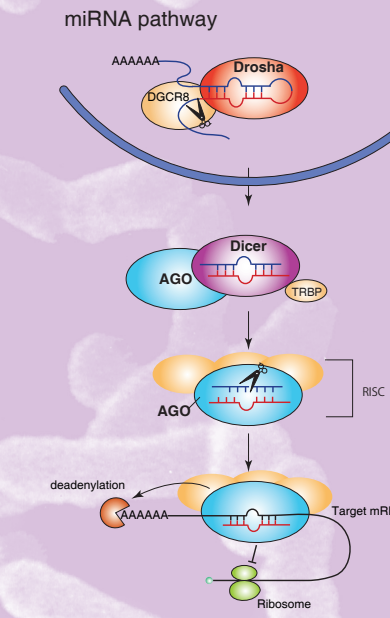


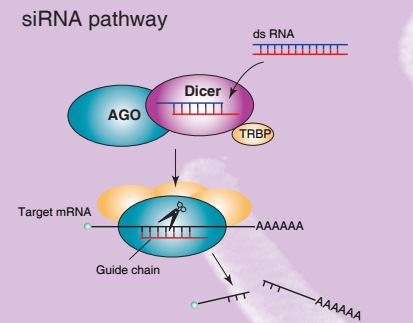
Small RNA

RNA interference (RNAi) is a mechanism that controls the expression of genes in a sequence specific manner. Small RNAs of 21-25 residues are incorporated into the RNA-induced signaling complex (RISC) and control the translation (and degradation) of their complementary mRNAs. miRNA and siRNA, both affect the translation rate of their target mRNA, small RNAs also include piRNA, which is involved in silencing of transposons.

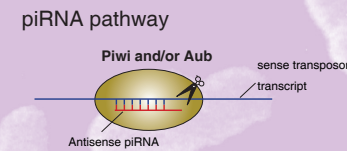
miRNA has recently been shown to play an important role in cell growth, development, differentiation and apoptosis. Changes in the expression of miRNAs are associated with various diseases, including cancer. It is important to identify the target mRNAs for understanding the disease mechanism.



miRNA initially forms a stem loop, which is cut the RNaseIII type nuclease Drosha to produce the precursor miRNA (pre-miRNA). Pre-miRNA is transported to the cytoplasm and cut into a double strand of 21-25 nucleotides by Dicer. The miRNA is then passed on to a member of the Argonaute protein family (AGO). The passenger strand of the double stranded miRNA is released, and the guide strand gets bound to the 3' untranslated end of the target mRNA. There, it influences the translation directly or by causing degradation of the targeted mRNA.



siRNA thought to be a defense mechanism against RNA virus. Dicer has RNase III activity and produces an siRNA duplex of 21-25 nucleotide length from long double stranded RNA (dsRNA) in the cytoplasm. The guide chain of the siRNA duplex gets taken up by AGO inside the RISC complex, where it gets attached to complementary mRNA sequences and silences those transcripts. The passenger chain exits the RISC complex and is degraded.



PIWI-interacting RNA (piRNA) are derived from single-stranded transposon transcripts. piRNA fragments are produced by the poorly understood "primary processing pathway", which does not require RNaseIII activity. piRNA is loaded onto PIWI, a family of proteins only expressed in germ cells, where they induce the cleavage of complementary transposon transcripts.

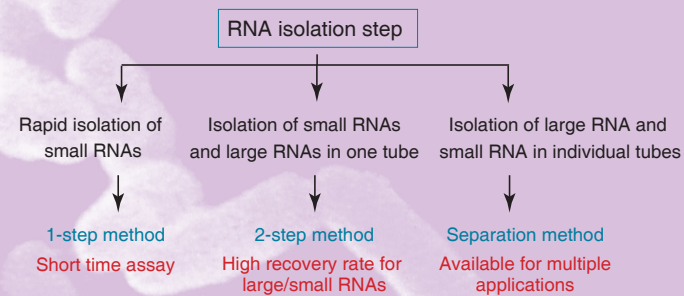
• RIP-Assay Kit for microRNA

Code No.	Product	Quantity
RN1005	RIP-Assay Kit for microRNA	10 assays

The RIP-Assay Kit for microRNA is optimized for the isolation of functionally related mRNAs and miRNAs by means of RNA binding protein immunoprecipitation (RIP).

The kit can be used to:

- Isolate small and large RNAs from a RiboCluster simultaneously (2-step method)
- Isolate small and large RNAs from a RiboCluster as separate fractions (separation method)
- Quickly isolate small RNAs from a RiboCluster (one-step method)
- No phenol is used in the RNA extraction step



Also available :

- RIP-Assay Kit (for mRNA)
- RIP-Certified Antibodies to over 50 RBPs, including Ago2, HuR, IMP1, eIF4E, etc.
- RiboTrap

Sold separately for use with either kit.

References

- 1) Shiomi, H., and Shiomi, MC. 2009. On the road to reading the RNA interference code. Nature 457: 396-404
- 2) Jinek M., and Doudna J. A. 2009. A three-dimensional view of the molecular machinery of RNA interference. Nature 457 : 405-412
- 3) Kawamata T., and Tomari Y. 2010. Making RISC. Trend in Biochem Sci 35: 368-376
- 4) Keene J.D. 2007. RNA regulons: coordination of post-transcriptional events. Nature Review 8: 533-543

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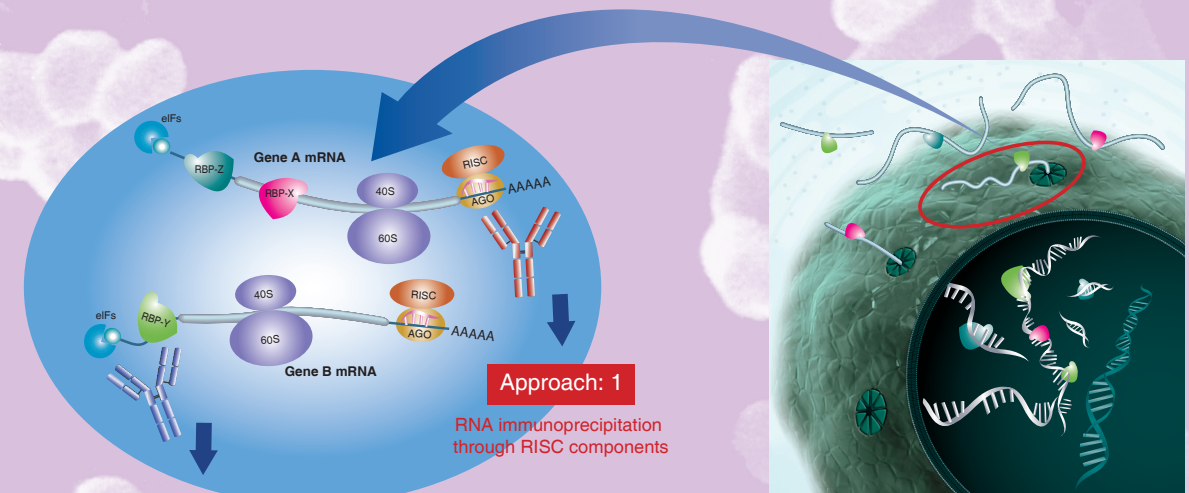
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RIP-Assay Kit for miRNA

- Analyze intact RBP-mRNA complexes
- Identify miRNAs bound to their mRNA targets
- Analyze the maturation mechanism of miRNA
- Identify RISC components and analyze their function
- Identify novel miRNAs and construct miRNA libraries



Isolate the mRNAs and miRNAs bound to your RBP cluster of interest

Identification



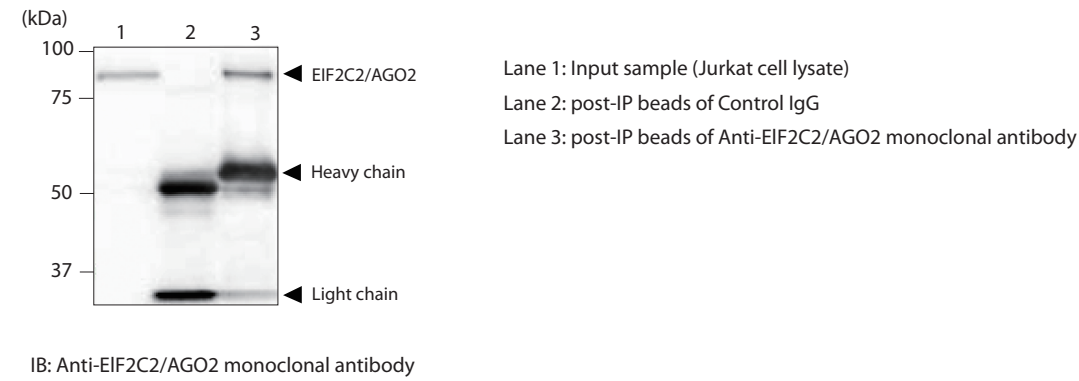
mRNA-miRNA matching

Understand the regulation of your mRNA and its protein product

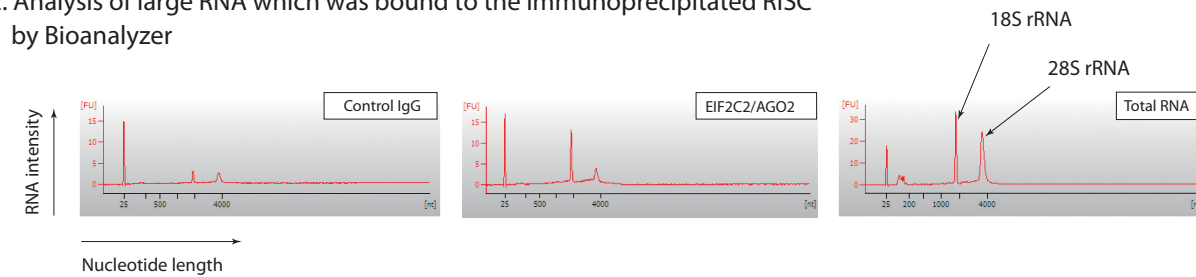
Recent studies suggest that mRNAs encoding functionally related proteins and their regulatory microRNAs bind to the common RNA binding proteins (RBPs), forming so-called RiboClusters. MBL's RIP-Assay Kit for micro RNA enables you to immunoprecipitate RiboClusters through RBP specific antibodies and isolate the RNAs and their regulatory micro RNAs associated with the RiboCluster (RIP assay). You can thus isolate cluster specific mRNAs as well as miRNAs for subsequent analysis by microarray, sequencing or RT-PCR.

RISC immunoprecipitation using Ago2 antibody (Approach 1)

1. Confirmation of Ago2 binding to beads after immunoprecipitation

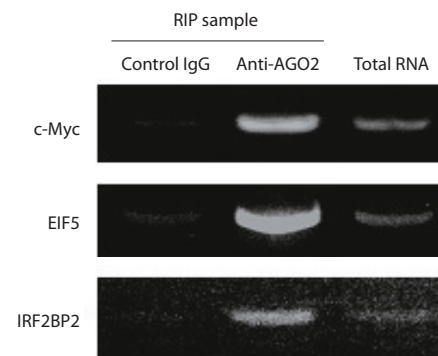


2. Analysis of large RNA which was bound to the immunoprecipitated RISC by Bioanalyzer



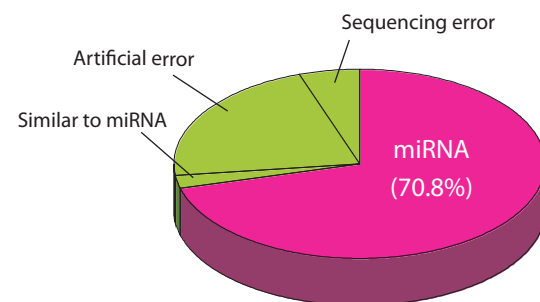
- an enrichment of large RNA was observed in the AGO cluster.

3. RT-PCR analysis of the isolated large RNA



- c-Myc, EIF5 and IRF2BP2 specific transcripts were enriched in the AGO2 immunoprecipitate

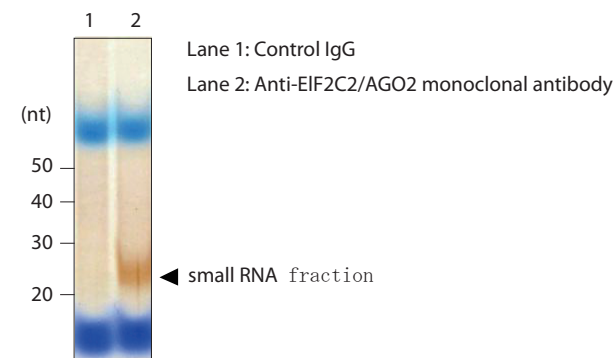
5. Sequence analysis of the purified small RNA



miRNA: coverage>90%, identity>90%
Similar to miRNA: coverage>80%, identity>80%
Artificial error: Errors induced by PCR cloning
Sequencing error: Errors related to the sequence read

- Many miRNAs were identified in the isolated small RNA. (96 clones analyzed).

4. Confirmation of isolated small RNA by silver staining



- small RNA was clearly enriched in the AGO2 immunoprecipitate.

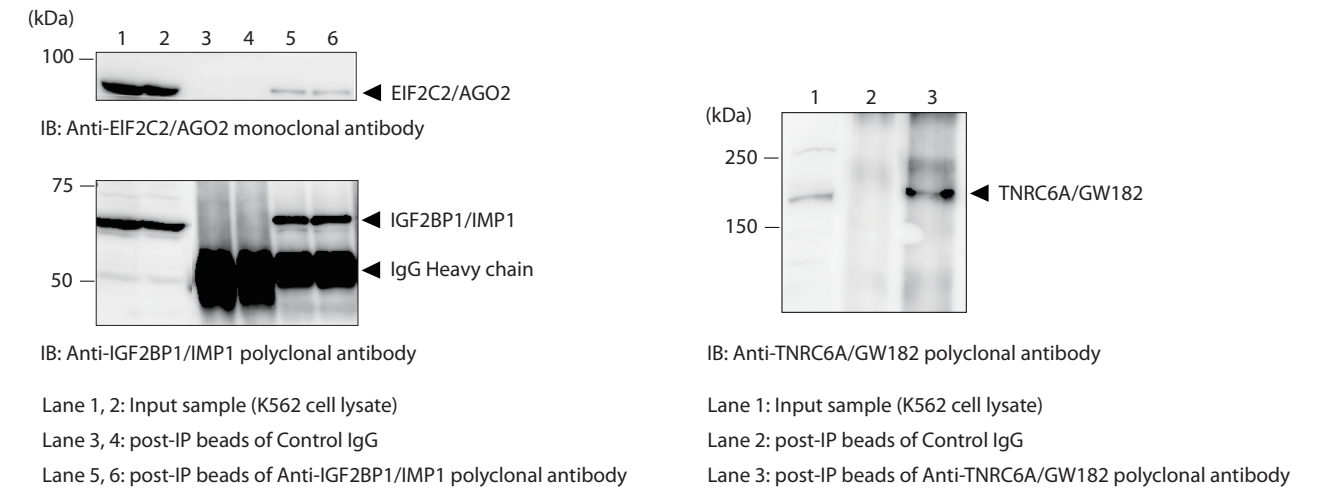
RIP Assay using antibodies against IMP1 and GW182

IGF2BP1/IMP1* is not part of the RISC complex (Approach 2)

TNRC6A/GW182 is part of the RISC complex (Approach 1)

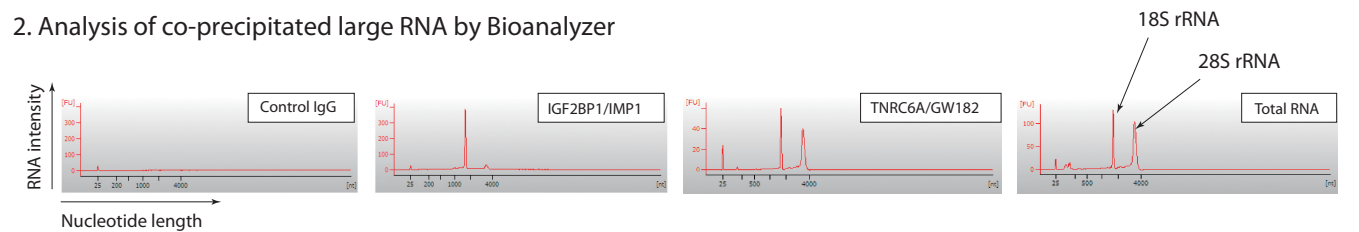
* IGF2BP1/IMP1 is reported to have a role on mRNA transport, distribution and regulation of translation.

1. Confirmation of RBP binding to beads after immunoprecipitation



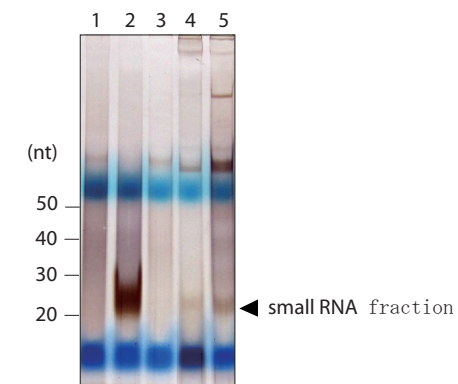
- The IMP1 antibody co-precipitated AGO2 as part of the IMP1 cluster. (This suggests that IMP1 and RISC were simultaneously bound to different regions of the same mRNA strand.)

2. Analysis of co-precipitated large RNA by Bioanalyzer



- an enrichment of large RNA was observed in both clusters.

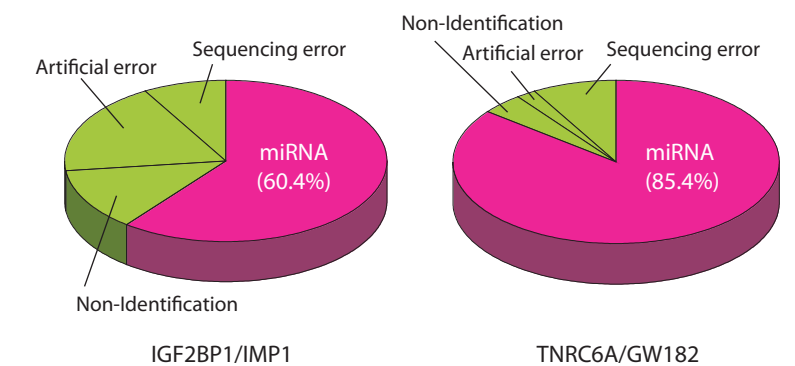
3. Confirmation of isolated small RNA by silver staining



Lane 1: Control IgG
Lane 2: Anti-EIF2C2/AGO2 monoclonal antibody
Lane 3: Control IgG
Lane 4: Anti-IGF2BP1/IMP1 polyclonal antibody
Lane 5: Anti-TNRC6A/GW182 polyclonal antibody

- small RNA was enriched in the RIP sample from each RBP antibody.

4. Sequence analysis of the purified small RNA



miRNA: (coverage>90%, identity>90%)
Non-Identification: Sequences which could not be identified (including novel miRNAs)
Artificial error: Errors induced by PCR cloning
Sequencing error: Errors related to the sequence read

- Many miRNAs were identified even in the RIP sample of IMP1, which is not a RISC component. (48 clones analyzed)