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For Research Use Only. Not for use in diagnostic procedures.

RiboCluster Profiler™

RIP-Assay Kit

10 assays

CODE No. RN1001

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I. Introduction

Please read these instructions carefully before beginning the assay.

It is significantly important to isolate "high-quality RNA" from various materials to validate experiments such as reverse transcription polymerase chain reaction (RT-PCR) and gene expression analysis based on microarray technology (Chip analysis) because experimental results may be sensitive to RNA quality. In order to obtain "high-quality RNA", and reduce the chance of RNase contamination, gloves should be worn when proceeding RIP-Assay, and RNase-free microcentrifuge tubes and pipette tips should be used for the assay.

1. Background and Introduction

Post-transcriptional regulation of gene expression is a ribonucleoprotein (RNP)-driven process, which involves RNA binding proteins (RBPs) and noncoding RNAs that regulate splicing, nuclear export, subcellular localization, mRNA stability and translation. This area has recently become the focus of many research groups and progress is being made using the yeast, *Saccharomyces cerevisiae* and various types of mammalian cell systems. Those observations have confirmed the posttranscriptional RNA operon concept in which mRNAs that encode functionally related proteins are coordinately regulated during cellular processes such as proliferation, differentiation or drug treatment. For example, mRNAs encoding proteins that function in a particular cell process or pathway can be found within a unique mRNP complex, which consists of mRNA and RNP. This provides valuable information regarding not only known components of a particular process or pathway, but importantly, leads to the identification of novel components representing potential therapeutic targets and biomarkers. In addition to those targets identified by pathway expansion, the specific RBPs regulating RNA functions may be potential therapeutic targets in their own right.

In order to understand posttranscriptional control of gene expression, RIP-Chip technologies that allow the isolation and identification of mRNAs, microRNAs and protein components of RNP complexes from cell extracts using antibodies to RBPs and microarrays have been developed.

2. Product Description

RIP-Assay Kit is optimized for performing the RIP-Chip process. In the RIP-Assay protocol, mRNP complexes are isolated from cell extracts by immunoprecipitation with *RIP-Certified Anti-RBP Antibodies* provided from MBL. mRNAs are isolated from mRNPs using guanidine hydrochloride. Thus, *RIP-Assay Kit* does not contain phenol or chloroform, allowing safe isolation of "high-quality RNA" from RNP complexes without degradation. Once purified, the RNAs present in the complex are analyzed to identify the target mRNAs using various molecular biology tools such as RT-PCR, gene expression analysis based on microarray technology (Chip analysis), or sequencing.

The major advantage of *RIP-Assay Kit* over most other omics approaches is that the majority of the RNAs identified exhibit structural and functional relationships. Structurally, the mRNAs in a complex contain common binding motifs for the RNA binding protein employed. Functionally, the RNAs identified

generally share a common RNA regulatory network as they tend to be co-localized by the RNA binding protein which may determine how they are utilized in the cell. A second advantage of *RIP-Assay Kit* over traditional RNA isolation and analysis methods is that the fractionation procedure effectively concentrates the RNA species bound to a specific binding protein enabling small changes in levels of low-abundance RNAs to be detected with a greatly increased signal-to-noise ratio. When performed according to the supplied protocol, another advantage of *RIP-Assay Kit* is that RNA reassociation is minimized and RNAs contained in the RNP complex of interest are abundantly recovered.

3. Kit Components

10 assays

1. Lysis Buffer	26 mL × 1 bottle
2. Wash Buffer	35 mL × 2 bottles
3. Normal Rabbit IgG	0.2 mL × 1 vial: Negative control: 200 μg of normal rabbit IgG in 200 μL of phosphate buffered saline (PBS) containing 50% Glycerol (pH 7.2).
4. High-Salt Solution	6 mL × 1 vial: In some cases, addition of this solution to both Lysis Buffer and Wash Buffer is required. Please refer to the datasheet of *RIP-Certified Antibody* (See **Related Products**).
5. Solution I	0.26 mL × 1 vial: enzyme solution
6. Solution II†	10 mL × 1 vial: diluent for Solution I
7. Solution III‡	7 mL × 1 vial: protein dissolvent Solution III can dissolve proteins and dissociate immunocomplex.
8. Solution IV	55 μL × 1 vial: co-precipitator Solution IV can increase RNA precipitation efficiently.

Note: † Solution II may become turbid when stored for long-term at 2-8ºC. Turbidity does not affect performance. If Solution II is turbid, equilibrate to room temperature (15-25ºC) and mix well before use.

‡ Precipitates may appear when Solution III is stored for long-term at 2-8ºC. If Solution III contains precipitates, dissolve them by equilibrating the solution to room temperature (15-25ºC) and mix well before use.

‡ This reagent contains guanidine hydrochloride; this is a potentially hazardous substance and should be used with appropriate caution.

4. Storage and Stability

RIP-Assay Kit is stable for 2 years from the date of manufacture when stored at 4ºC. Do not freeze.

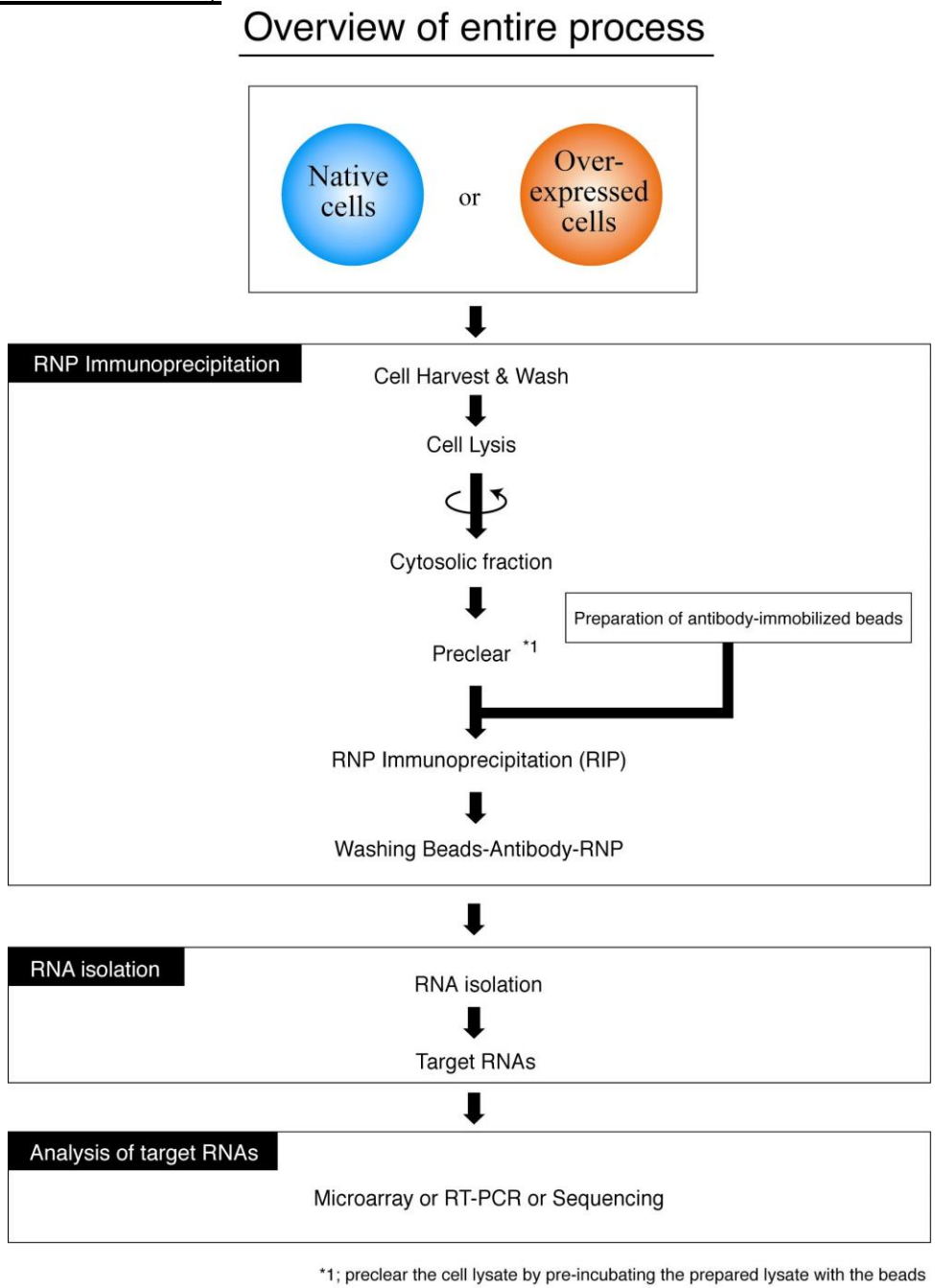
5. Materials Required but Not Provided

1. RIP-Certified Antibody (See **Related Products**)
2. Microcentrifuge capable of 15,000 × *g*
3. Microcentrifuge tube (1.5 mL or 2 mL) (Nuclease-free)
   (Recommendation; use low-adhesion tube for RIP-Assay)
4. Centrifuge capable of 2,000 × *g*
5. Centrifuge tube (15 mL or 50 mL)
6. Pipettes (5 mL, 10 mL, 25 mL) (Nuclease-free)
7. Pipette tips (10 μL, 20-100 μL, 200 μL, and 1,000 μL) (Nuclease-free)
   (Recommendation; use low-adhesion pipette tip for RIP-Assay)
8. Ultra-low temperature freezer (-80ºC)
9. Freezer (below -20ºC)
10. End-over-end rotator
11. Vortex mixer
12. Gloves
13. Protease inhibitor (molecular biology grade)*
    Commercial reagent
    Aprotinin
    Leupeptin
    Phenylmethylsulfonyl fluoride (PMSF)
14. RNase inhibitor*
15. Dithiothreitol (DTT)*
16. Protein A or Protein G Agarose beads**
17. 100% Ethanol (molecular biology grade)
18. 100% 2-Propanol (molecular biology grade)
19. Nuclease-free PBS
20. Nuclease-free water
21. Isotype control IgG (if necessary)***

Note: * Recommended concentration of each reagent is shown in **Appendix**.
** Commercially available reagents confirmed to work with *RIP-Assay Kit* are shown in **Appendix**.
*** In the case of using monoclonal antibodies for RNP immunoprecipitation, the isotype control IgG should be prepared as a negative control. Please refer to **Related Products**.

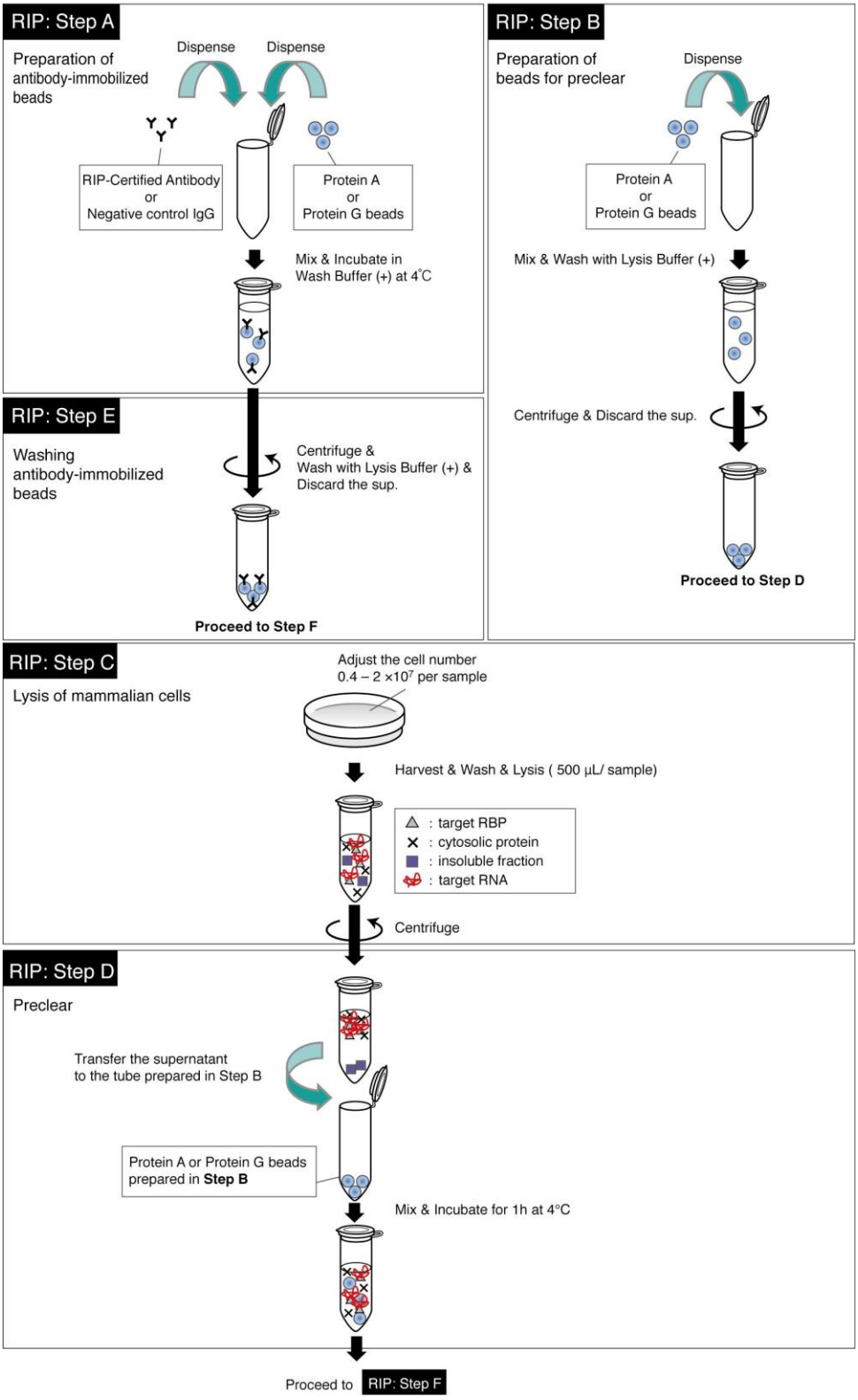
II. RIP-Assay Kit Procedure

1. Procedure Summary

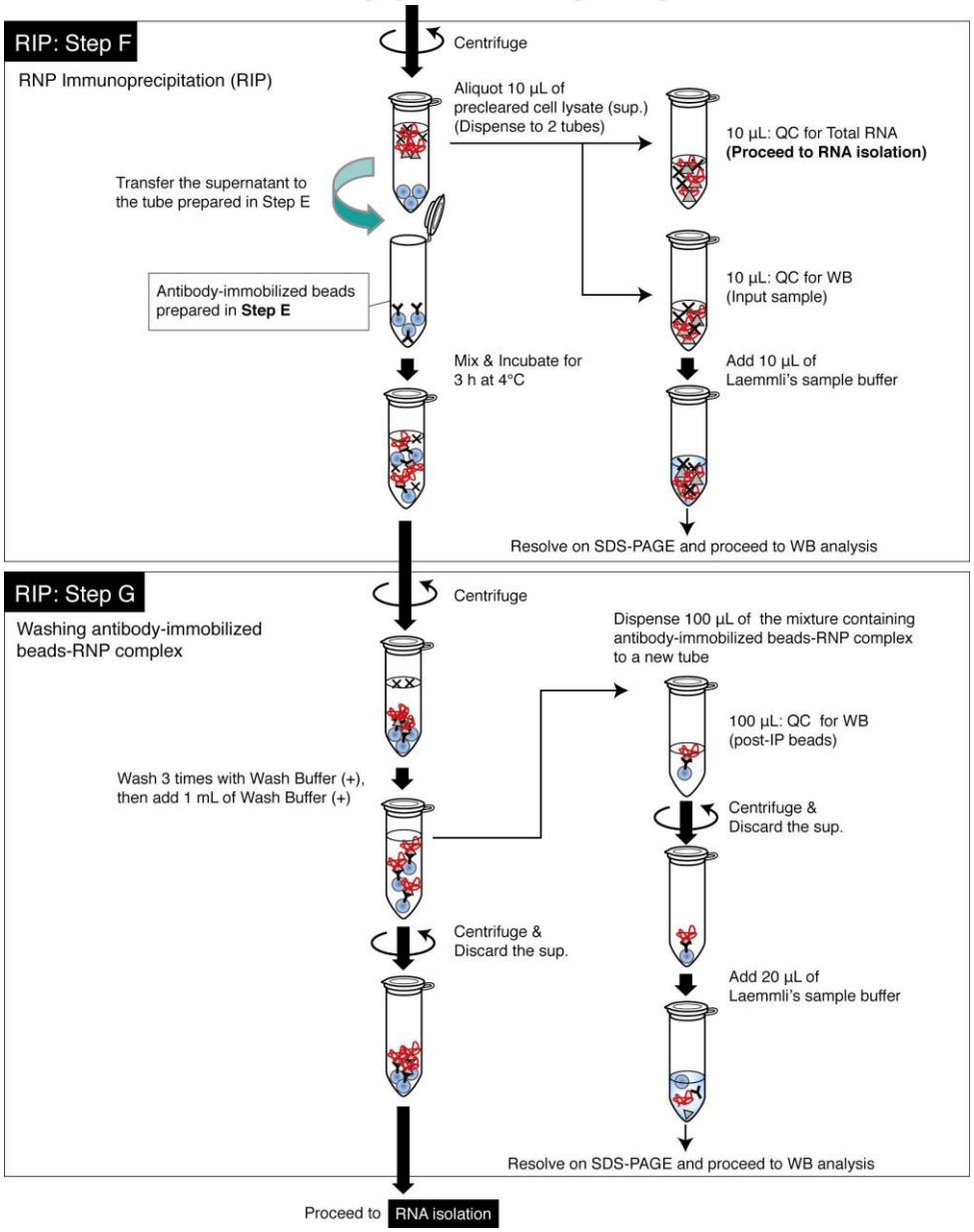


*1; preclear the cell lysate by pre-incubating the prepared lysate with the beads

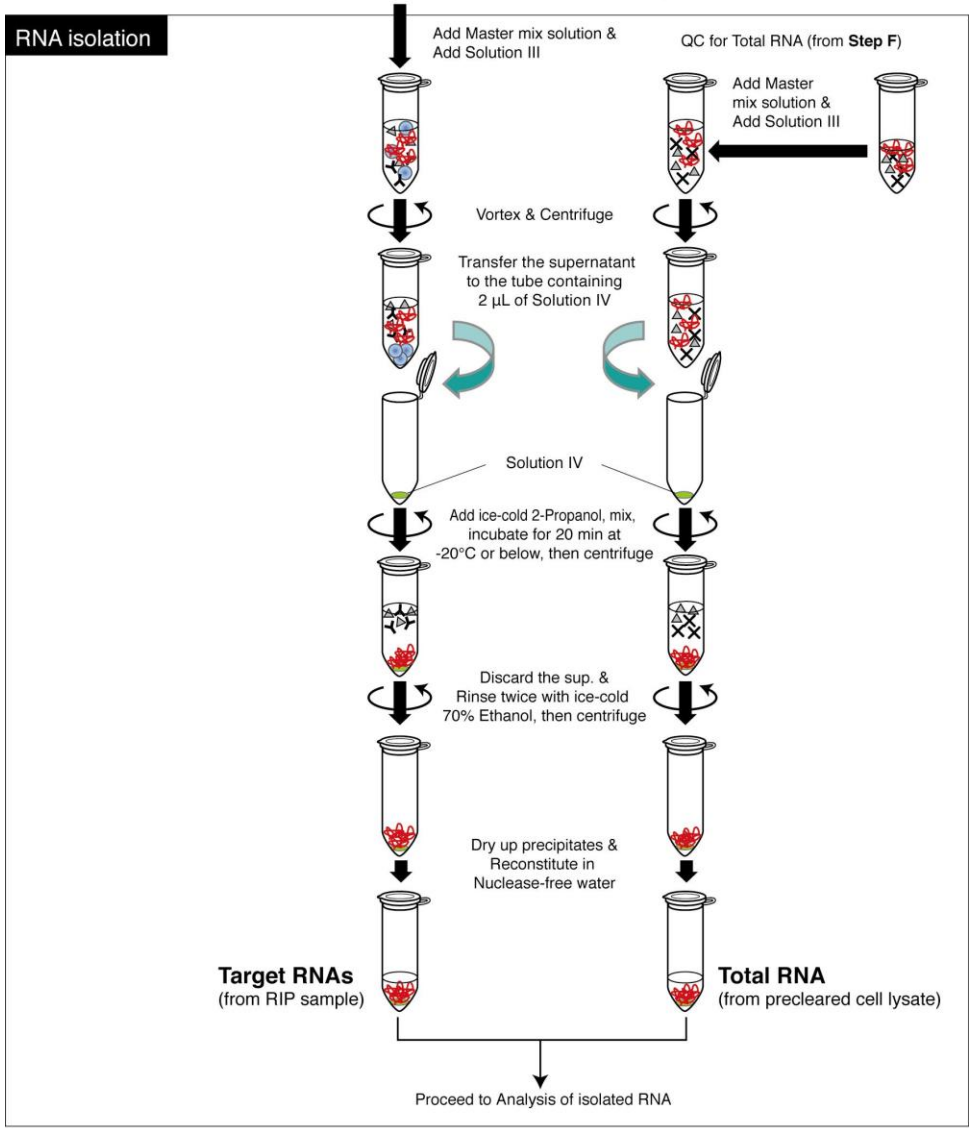
RIP-Assay process by step

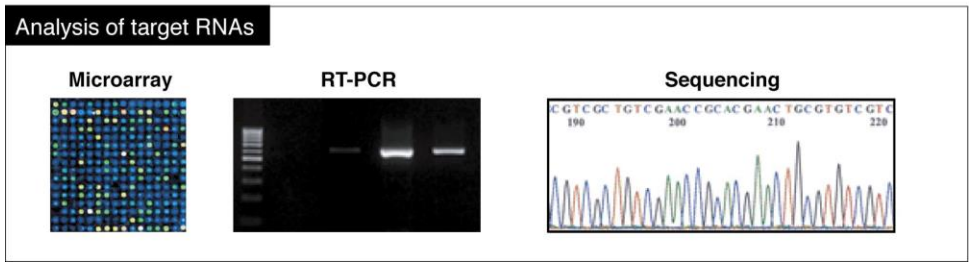


RIP-Assay process by step-cont.



RIP-Assay process by step-cont.





2. Buffer Preparation

1. Lysis Buffer

Add appropriate concentrations of protease inhibitors, RNase inhibitor, and dithiothreitol (DTT) to Lysis Buffer just before use. Lysis Buffer containing these reagents is described as Lysis Buffer (+) in the following protocols. The optimal concentration of each reagent for RIP-Assay is shown in **Appendix**.

2. Wash Buffer

Add appropriate concentration of dithiothreitol (DTT) to Wash Buffer just before use. Wash Buffer containing DTT is described as Wash Buffer (+) in the following protocols. The optimal concentration of the reagent for RIP-Assay is shown in **Appendix**.

(Precaution: Additional Buffer Preparation)

In some cases, both the Lysis Buffer (+) and Wash Buffer (+) may require the addition of appropriate volumes of High-Salt Solution (in these cases, add 30 µL of High-Salt Solution to each mL of Lysis Buffer and Wash Buffer). Please refer to the datasheet of *RIP-Certified Antibody* (See **Related Products**).

3. Protocols For RNP Immunoprecipitation Assay (RIP-Assay)

The following protocol is for the isolation of RNA from the RNP complex expressed in various cells. The expression level of target RBP may vary. If necessary, adjust the number of cells used for this assay between 4 million to 20 million per sample.

◆ RNP Immunoprecipitation (RIP)

(A. Pre-step: Preparation of Antibody-immobilized Protein A or Protein G Agarose beads)

1. Wash the Protein A or Protein G agarose beads 3 times with equal amount of nuclease-free PBS (centrifuge; 2,000 × *g* for 1 minute at 4ºC).
2. Aliquot 25 µL of the 50% beads slurry to each new microcentrifuge tube.
3. Add 1 mL of Wash Buffer (+) to each tube.
4. Add 15 µg of Antibody (Normal Rabbit IgG as a negative control or *RIP-Certified Antibody* for target RBP, respectively) to each tube.
5. Incubate the tube with rotation for at least 30 minutes at 4ºC. If necessary, this incubation can be extended to overnight.

(B. Pre-step: Preparation of Protein A or Protein G Agarose beads for preclear)

6. Wash the Protein A or Protein G agarose beads 3 times with equal amount of nuclease-free PBS (centrifuge; 2,000 × *g* for 1 minute at 4ºC).
7. Aliquot 25 µL of the 50% beads slurry to each new microcentrifuge tube.
8. Add 500 µL of Wash Buffer (+) to each tube, and mix briefly.
9. Centrifuge the tube at 2,000 × *g* for 1 minute at 4ºC.
10. Discard the supernatant carefully.
11. Leave the beads at 4ºC or on ice until starting **Preclear step**.
12. Just before **Preclear step**, wash the beads once with 500 µL of Lysis Buffer (+).
13. Centrifuge the tube at 2,000 × *g* for 1 minute at 4ºC.

14. Discard the supernatant carefully. Use these Protein A or Protein G agarose beads washed once with Lysis Buffer (+) for **preclear step** (step 28).

(C. Lysis of Mammalian Cells)

Note: In order to obtain "high-quality RNA", freshly cultured cells should be used in RIP-Assay.

15. Detach the cells from the culture dish by pipetting or using a cell scraper, if necessary. Collect the cell suspension into centrifuge tube.
16. Centrifuge the cell suspension at 300 × *g* for 5 minutes at 4ºC to pellet the cells. Carefully remove and discard the supernatant.
17. Wash the cells by resuspending the cell pellet with ice-cold PBS.
18. Centrifuge the cell suspension at 300 × *g* for 5 minutes at 4ºC to pellet the cells. Carefully remove and discard the supernatant.
19. Wash the cells once again using steps 17-18.
20. Wash the cells by resuspending the cell pellet with ice-cold nuclease-free PBS.
21. Centrifuge the cell suspension at 300 × *g* for 5 minutes at 4ºC to pellet the cells. Carefully remove and discard the supernatant.
22. Wash the cells by resuspending the cell pellet with ice-cold nuclease-free PBS.
23. Aliquot the cell suspension to each new microcentrifuge tube.
24. Centrifuge the cell suspension at 300 × *g* for 5 minutes at 4ºC to pellet the cells. Carefully remove and discard the supernatant.
25. Add 500 µL of Lysis Buffer (+) to each tube containing the cell pellet, and vortex thoroughly.
26. Incubate the tube for 10 minutes at 4ºC or on ice.
27. Centrifuge the cell suspension at 12,000 × *g* for 5 minutes at 4ºC.

(D. Preclear step)

28. Transfer the supernatant (cell lysate) to the tube (prepared in step 14) containing Protein A or Protein G agarose beads washed once with Lysis Buffer (+); that were prepared in steps 6-14.
29. Incubate the tube with rotation for 1 hour at 4ºC.

(E. Washing the Antibody-immobilized Protein A or Protein G Agarose beads)

During **Preclear step**, wash once the Antibody-immobilized Protein A or Protein G agarose beads with 1 mL of Lysis Buffer (+).

30. Centrifuge the tube (prepared in step 5) containing Antibody-immobilized Protein A or Protein G agarose beads at 2,000 × *g* for 1 minute at 4ºC.
31. Discard the supernatant carefully.
32. Add 1 mL of Lysis Buffer (+), and mix briefly, then centrifuge the tube at 2,000 × *g* for 1 minute at 4ºC.
33. Discard the supernatant carefully.

(F. Preparation of Antibody-immobilized Protein A or Protein G Agarose beads-RNP complex)

34. Centrifuge the tube (prepared in step 29) containing cell lysate and Protein A or Protein G agarose beads at 2,000 × *g* for 1 minute at 4ºC.

Note*: Preparation of Quality Check (QC) sample

In order to confirm whether RIP-Assay is running properly, we recommend to perform quality check. Collect QC sample and check the protein and RNA expression level at some steps. At least two additional aliquots may be retained for quality check. Use one of the aliquots (10 µL of precleared cell lysate, Input sample) for analysis of RBP expression level by Western blotting, and use the other aliquots (10 µL of precleared cell lysate) for analysis of Total RNA (See **Example of RIP-Assay Results**).

- **Preparation of Input sample (for Western blotting)**
  - i) Add 10 µL of Laemmli's sample buffer to 10 µL of precleared cell lysate, boil for 3-5 minutes, mix well, and centrifuge.
  - ii) Resolve 20 µL of the prepared sample on SDS-PAGE, and proceed to Western blotting analysis.
- **Preparation of Total RNA (for quality check of Total RNA)**
  - i) Place 10 µL of precleared cell lysate at -80ºC until beginning of RNA isolation.
  - ii) After RNP immunoprecipitation, use the lysate to prepare Total RNA sample according to **RNA isolation protocol** (See below).

35. Transfer 500 µL of the precleared cell lysate to the tube (prepared in step 33) containing Antibody-immobilized Protein A or Protein G agarose beads washed once with Lysis Buffer (+); that were prepared in steps 30-33.
36. Incubate the tube with rotation for 3 hours at 4ºC.

(G. Wash of Antibody-immobilized Protein A or Protein G Agarose beads-RNP complex)

37. Centrifuge the tube (prepared in step 36) containing Antibody-immobilized Protein A or Protein G agarose beads-RNP complex at 2,000 × *g* for 1 minute at 4ºC.
38. Discard the supernatant carefully.
39. Add 1 mL of Wash Buffer (+), mix briefly, and centrifuge the tube at 2,000 × *g* for 1 minute at 4ºC.
40. Discard the supernatant carefully.
41. Wash the Antibody-immobilized beads-RNP complex twice using steps 39-40.
42. For fourth wash, add 1 mL of Wash Buffer (+), then mix well and dispense 100 µL of the mixture to new microcentrifuge tube for QC sample (post-IP beads). Use those aliquots for quality check by Western Blotting (See **Example of RIP-Assay Results**).

Note*: Preparation of QC sample (for post-IP beads)

- **Preparation of post-IP beads sample (for Western blotting)**
  - i) Centrifuge the tube containing 100 µL of the mixture at 2,000 × *g* for 1 minute at 4ºC.
  - ii) Discard the supernatant carefully.
  - iii) Resuspend the precipitated beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, mix well and centrifuge the tube at 2,000 × *g* for 1 minute.
  - iv) Resolve 20 µL of the prepared sample on SDS-PAGE, and proceed to Western blotting analysis.

43. Centrifuge the tube containing Antibody-immobilized Protein A or Protein G agarose beads-RNP complex at 2,000 × *g* for 1 minute at 4ºC.
44. Discard the supernatant carefully.

◆ RNA Isolation

(from Antibody-immobilized Protein A or Protein G agarose beads-RNP complex)

Solution II and Solution III should be equilibrated to room temperature before use.
Reagents should be briefly but thoroughly mixed before use.

1. Prepare Master mix solution by diluting 10 μL of Solution I with 390 μL of Solution II per sample.
2. Dispense 2 μL of Solution IV to each new microcentrifuge tube for step 5.
3. Add 400 μL of Master mix solution to each tube (prepared in RIP-step 44) containing Antibody-immobilized Protein A or Protein G agarose beads-RNP complex (obtained in previous **RNP Immunoprecipitation**), vortex thoroughly, then spin-down.
4. Add 250 μL of Solution III to each tube, vortex thoroughly, then centrifuge the tube at 2,000 × *g* for 2 minutes at room temperature.
5. Carefully transfer the supernatant to the tube containing 2 μL of Solution IV prepared in step 2. (Avoid to remove the Protein A or Protein G agarose beads from the pellet. Contamination of the beads may affect following steps.)
6. Add 600 μL of ice-cold 2-propanol to each tube, vortex briefly but thoroughly, then spin-down.
7. Incubate the tube at -20ºC or below for 20 minutes (or overnight, if necessary).
8. Centrifuge the tube at 12,000 × *g* for 10 minutes at 4ºC, then aspirate the supernatant carefully.
9. Rinse the pellet with 500 μL of ice-cold 70% ethanol, and mix briefly.
10. Centrifuge the tube at 12,000 × *g* for 3 minutes at 4ºC, then aspirate the supernatant carefully.
11. Rinse the pellet once again using steps 9-10.
12. Dry up the pellet by aspirating excess ethanol followed by evaporation for 5-15 minutes at room temperature. Avoid RNase contamination. (Evaporation in clean bench is recommended.)
13. Reconstitute the pellet in 20 μL of nuclease-free water.
14. Store at -80ºC until starting following analysis.

In order to obtain QC sample, isolate Total RNA from 10 μL of precleared cell lysate (prepared in RIP-step 34) according to above steps 1-14.

Additional Procedure: Analysis of isolated RNA

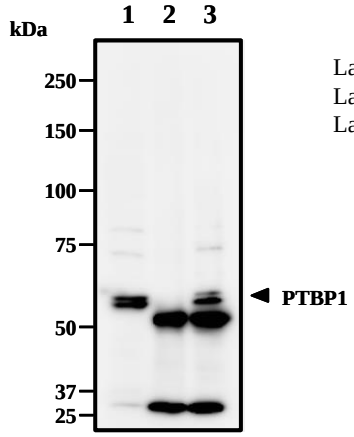
We recommend qualitative and quantitative analysis of isolated RNAs prior to downstream analysis such as RT-PCR, microarray and sequencing. These technologies may be useful for profiling RNAs in the target mRNP complex.

- **Quality control for isolated RNAs**

  Quantify the isolated RNAs with NanoDrop (Thermo Fisher Scientific Inc.), and characterize the RNAs with Bioanalyzer (Agilent Technologies, Inc.). It is very important for comprehensive analysis such as microarray to retain high-quality RNA because experimental results may be sensitive to RNA quality.

III. Example of RIP-Assay Results

1. Quality check: Analysis of RBP expression level by Western blotting.

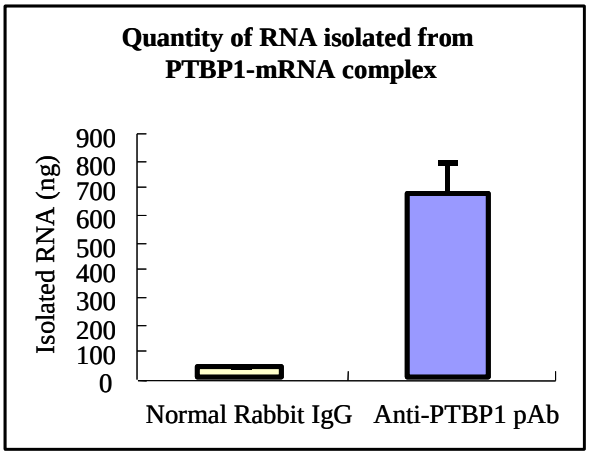


Lane 1: Input sample (Precleared cell lysate, Protocol step 34)
Lane 2: post-IP beads of Normal Rabbit IgG, Protocol step 42
Lane 3: post-IP beads of Anti-PTBP1 polyclonal antibody (Code No. RN011P), Protocol step 42

Western blotting (IB: *Anti-PTBP1 polyclonal antibody*, Code No. RN011P)

Quality check of immunoprecipitated endogenous PTBP1 expressed in Jurkat cells. 10 µL of precleared cell lysate (Input sample, Protocol step 34) contained detectable level of target RBP (PTBP1) (Lane 1). The target RNP complex was successfully concentrated by RIP-Assay because no PTBP1 was detected in the post-IP beads coated with Normal Rabbit IgG, but PTBP1 was detected in the post-IP beads coated with anti-PTBP1 polyclonal antibody (lanes 2 and 3, respectively).

2. Quality check: Quantification of isolated RNA with NanoDrop.



Average quantity of isolated RNA (n=2)

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

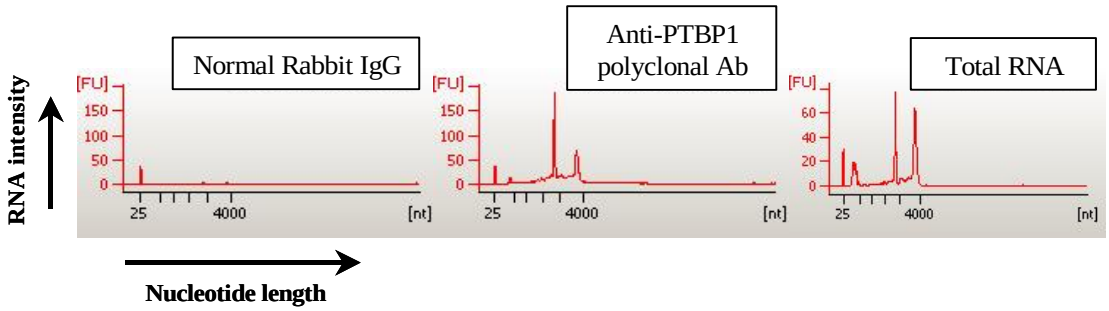
Note: Quantity of RNA was calculated based on volume ratio used for RNA isolation. Quantity of Total RNA represents whole amount of RNA in precleared cell lysate.

Quantification of isolated RNA with NanoDrop

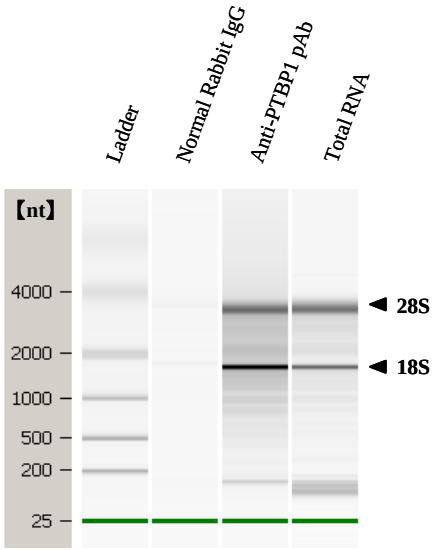
The RNA isolated from the endogenous PTBP1-mRNA complex expressed in Jurkat cells was quantified with a spectrophotometer (NanoDrop) according to manufacturer's instructions (Thermo Fisher Scientific Inc.). In comparison with the quantity of RNA isolated from the Normal Rabbit IgG complexes (negative control), the RNA obtained from the anti-PTBP1 polyclonal antibody-immunoprecipitates was significantly enriched.

3. Quality check: Characterization of isolated RNA with Bioanalyzer.

a)



b)



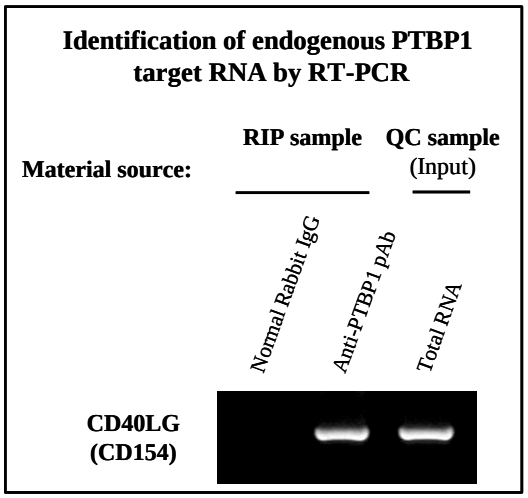
a) Migration profile of isolated RNA

b) Gel electrophoresis image of isolated RNA

Characterization of isolated RNA with Bioanalyzer

Cellular PTBP1-associated RNA in Jurkat cells was isolated by *RIP-Assay Kit* and *RIP-Certified Anti-PTBP1 polyclonal antibody* (Code No. RN011P). Endogenous PTBP1-associated RNA was analyzed on a Bioanalyzer RNA pico chip (Agilent Technologies, Inc.) according to manufacturer's instructions. RNA isolated from the PTBP1 complex containing mRNP showed a different migration profile compared with that isolated from the Normal Rabbit IgG complex (negative control). Total RNA was also isolated from Jurkat cells. The migration profile of the Total RNA sample showed 2 main peaks at around 2,000 and 4,000 nucleotides corresponding to 18S and 28S ribosomal RNA, respectively.

4. Identification of target RNA isolated from cellular RNP complex by RT-PCR.



Identification of endogenous PTBP1 target RNA by RT-PCR

The association of endogenous PTBP1 with endogenous target RNA (in this case, CD40LG) in Jurkat cells was tested by RIP-Assay, followed by detection of the target transcripts of interest by RT-PCR of RIP materials. PCR products were visualized by electrophoresis in ethidium bromide-stained 2% agarose gels to ensure correct size.

Identification of target RNA isolated from cellular PTBP1 containing mRNPs by RT-PCR

Cellular PTBP1-associated RNA in Jurkat cells was isolated by *RIP-Assay Kit* and *RIP-Certified Anti-PTBP1 polyclonal antibody* (Code No. RN011P). An equal amount of Normal Rabbit IgG was used as a negative control. RNA in the RIP products was analyzed for the presence of specific target mRNA (for example, CD40LG) by RT-PCR using gene-specific primer pairs. Compared with Normal Rabbit IgG, the expression level of the PTBP1-target CD40LG mRNA in the anti-PTBP1 polyclonal antibody-immunoprecipitates was significantly enriched. Therefore, *RIP-Assay Kit* is useful for profiling mRNAs in the target RNP complex.

IV. Related Products

RIP-Assay Kit *for microRNA*

RN1005	RIP-Assay Kit *for microRNA* (10 assays)

RIP-Certified Antibody

RN001P	Anti-EIF4E pAb (polyclonal)
RN002P	Anti-EIF4G1 (Human) pAb (polyclonal)
RN003P	Anti-EIF4G2 pAb (polyclonal)
RN004P	Anti-ELAVL1 (HuR) pAb (polyclonal)
RN005P	Anti-ELAVL2 (HuB) (Human) pAb (polyclonal)
RN006P	Anti-ELAVL3 (HuC) pAb (polyclonal)
RN007P	Anti-IGF2BP1 (IMP1) pAb (polyclonal)
RN008P	Anti-IGF2BP2 (IMP2) pAb (polyclonal)
RN009P	Anti-IGF2BP3 (IMP3) pAb (polyclonal)
RN010P	Anti-MSI1 (Musashi1) pAb (polyclonal)
RN011P	Anti-PTBP1 (Human) pAb (polyclonal)
RN012P	Anti-STAU1 (Human) pAb (polyclonal)
RN013P	Anti-STAU2 (Human) pAb (polyclonal)
RN014P	Anti-TIA1 pAb (polyclonal)
RN015P	Anti-YBX1 pAb (polyclonal)
RN019P	Anti-HNRNPK pAb (polyclonal)
RN020P	Anti-ILF3 (Human) pAb (polyclonal)
RN021P	Anti-KHDRBS1 pAb (polyclonal)
RN022P	Anti-PABPC4 pAb (polyclonal)
RN024P	Anti-PCBP1 pAb (polyclonal)
RN025P	Anti-PCBP2 pAb (polyclonal)
RN026P	Anti-PUM1 pAb (polyclonal)
RN027P	Anti-PUM2 pAb (polyclonal)
RN028P	Anti-EIF2C1 (AGO1) pAb (polyclonal)
RN032P	Anti-CIRBP pAb (polyclonal)
RN033P	Anti-TNRC6A (GW182) (Human) pAb (polyclonal)
RN037P	Anti-AUH pAb (polyclonal)
RN038P	Anti-CPEB1 pAb (polyclonal)
RN041P	Anti-KHDRBS2 (SLM1) pAb (polyclonal)
RN045P	Anti-SLBP pAb (polyclonal)
RN001M	Anti-IGF2BP1 (IMP1) mAb (6H6)
RN003M	Anti-EIF2C2 (AGO2) (Human) mAb (1B1-E2H5)
RN004M	Anti-Ribosomal P0/P1/P2 mAb (9D5)
RN005M	Anti-EIF2C2 (AGO2) mAb (2A8)
RN006M	Anti-EIF4E mAb (C107-3-5)
RN007M	Anti-ELAVL1 (HuR) mAb (C67-1)
RN009M	Anti-PABPC1 mAb (10E10)

Isotype Control Antibody

Various isotype control antibodies for mouse and rat are available.
Please visit our website at http://ruo.mbl.co.jp/je/rip-assay/

RBP Antibody

RBP Antibody works on WB and/or IP, but not certified for working on RIP-Assay.

RN023PW	Anti-PABPN1 pAb (polyclonal)
RN029PW	Anti-EIF2C2 (AGO2) pAb (polyclonal)
RN030PW	Anti-DICER1 pAb (polyclonal)
RN046PW	Anti-SYNCRIP (HNRNPQ) pAb
RN047PW	Anti-PTBP2 pAb (polyclonal)
RN051PW	Anti-HDLBP (Vigilin) pAb (polyclonal)
RN079PW	Anti-SRSF7 (9G8) pAb (polyclonal)
RN080PW	Anti-SRSF3 (SRp20) pAb (polyclonal)
RN081PW	Anti-SRSF9 (SRp30c) pAb (polyclonal)
RN082PW	Anti-SRSF5 (SRP40) pAb (polyclonal)
RN101PW	Anti-FBL (Fibrillarin) pAb (polyclonal)
RN102PW	Anti-GEMIN2 (Human) pAb (polyclonal)
RN106PW	Anti-SFPQ (PSF) pAb (polyclonal)
RN107PW	Anti-TARDBP (TDP-43) pAb (polyclonal)
RN113PW	Anti-DHX36 (RHAU) pAb (polyclonal)
RN114PW	Anti-HNRNPA1 pAb (polyclonal)
RN116PW	Anti-DDX39B (UAP56) pAb (polyclonal)
RN117PW	Anti-CCAR2 (DBC1) pAb (polyclonal)
RN121PW	Anti-FTO (Human) pAb (polyclonal)
RN129PW	Anti-DDX6 (RCK/p54) pAb (polyclonal)
RN002MW	Anti-CUGBP1 mAb (3A2)
RN008MW	Anti-ELAVL1 (HuR) mAb (C54-6)

Other Anti-RBP Antibodies are also available.
Please visit our website at http://ruo.mbl.co.jp/je/rip-assay/

V. Appendix

The following commercially available reagents have been confirmed to work with *RIP-Assay Kit* at the indicated final concentration.

Protease inhibitor		**Final concentration**
	Aprotinin	10 μg/mL
	Leupeptin	5 μg/mL
	PMSF	0.5 mM

Reducing agent		**Final concentration**
	DTT	1.5 mM

RNase inhibitor		**Distribution source**	**Code No.**	**Final concentration**
	RNase OUT	Invitrogen	10777-019	50−200 U/mL

Protein A beads		**Distribution source**	**Code No.**
	Protein A Sepharose CL-4B	GE Healthcare	17-0780-01

Protein G beads		**Distribution source**	**Code No.**
	Immobilized Protein G Plus	Pierce	22852

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