

RiboCluster Profiler™

RIP-Certified Antibody

Anti-PTBP1

Code No.	Quantity	Concentration	Form
RN011P	200 µL	1 mg/mL	Affinity Purified

BACKGROUND: The polypyrimidine tract binding protein 1 (PTBP1), a 57 kDa RNA binding protein also known as hnRNP1, has been implicated in multiple aspect of mRNA metabolism, including regulation of alternative splicing, internal ribosome entry site-driven translation, mRNA localization, and polyadenylation. Four of tandem RNA recognition motif domains give PTBP1 a strong binding activity to RNA. Posttranscriptional regulation of gene expression by PTBP1 has been reported on many genes, including CD154, insulin, VEGF and Human inducible nitric oxide synthase.

RIP-CERTIFIED ANTIBODY:

Posttranscriptional regulation of gene expression is a ribonucleoprotein-driven process, which involves RNA binding proteins (RBPs) and non-coding RNAs that affect splicing, nuclear export, subcellular localization, mRNA decay and translation. The RNP Immunoprecipitation-Chip (RIP-Chip), RIP-Seq and RIP-RTPCR allow the identification of multiple RNA targets of RBPs globally and within the context of a cell extract. Antibodies specific to the RNA binding protein of interest are used to co-immunoprecipitate the RNA binding protein and the associated subset of mRNAs. The mRNA content is interrogated using standard microarray or sequencing technology. RIP-Certified Antibody is validated for use in RNP Immunoprecipitation (RIP) in conjunction with the RIP-Assay Kit distributed from MBL. Its ability to immunoprecipitate mRNAs and RBPs complex was confirmed by quantitative and qualitative analysis on NanoDrop, Bioanalyzer and RT-PCR or microarray.

SOURCE: This antibody was purified from rabbit serum by affinity column chromatography. The rabbit was immunized with KLH conjugated synthetic peptide, KFKGDSRSAGVPSR corresponding to 46-59 aa.

FORMULATION: 200 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

INTENDED USE:

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

REACTIVITY: This antibody reacts with human PTBP1 (~57 kDa) on Western blotting, Immunoprecipitation and RNP Immunoprecipitation.

APPLICATIONS:

RNP Immunoprecipitation; 15 µg/500 µL of cell extract from 6×10^6 cells

Western blotting; 1:1,000 for chemiluminescence detection system

Immunoprecipitation; 5 µg/250 µL of cell extract from 2.5×10^6 cells

Immunohistochemistry; Not tested

Immunocytochemistry; Not tested

Flow cytometry; Not tested

Detailed procedure is provided in the following **PROTOCOLS.**

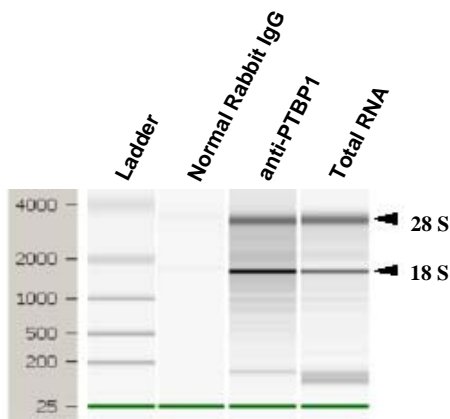
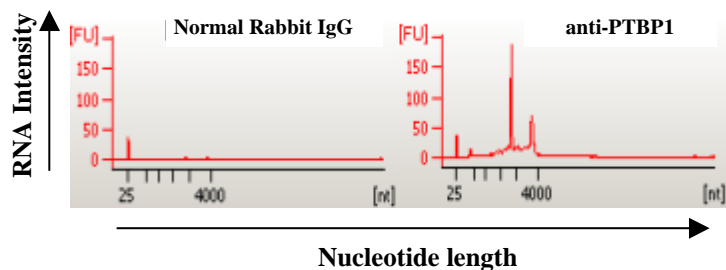
REFERENCES:

- 1) Porter, J. F., *et al.*, *J. Immunol.* **181**, 3336-3345 (2008)
- 2) Pautz, A., *et al.*, *J. Biol. Chem.* **281**, 32294-32302 (2006)
- 3) Cornelis, S., *et al.*, *Nucleic Acids Res.* **33**, 3095-3108 (2005)

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster
Cells	K562, 293T, HeLa, Jurkat	NIH/3T3, WR19L	Rat1	CHO
Reactivity on WB	+	-	-	-

LICENSING OPPORTUNITY: The RIP-Assay uses patented technology (US patent No. 6,635,422, US patent No. 7,504,210) of Ribonomics, Inc. MBL manufactures and distributes this product under license from Ribonomics, Inc. Researchers may use this product for their own research. Researchers are not allowed to use this product or RIP-Assay technology for commercial purpose without a license. For commercial use, please contact us for licensing opportunities at RIP@mbi.co.jp



Analysis of isolated RNA with Bioanalyzer.

Average of the RNA Quantity (n=2)	
Antibody	RNA (ng)
Normal Rabbit IgG	51.0
anti-PTBP1	678.0
Total RNA	84570.0

PROTOCOLS:

RNP Immunoprecipitation

Some buffer and reagents are included in the RIP-Assay Kit (code. RN1001). Please also refer to the protocol packaged in the RIP-Assay Kit.

[Material Preparation]

1. Lysis Buffer (+)
Before using the Lysis Buffer, protease inhibitors, RNase inhibitors, and DTT are added to the Lysis Buffer at the appropriate concentration.
2. Wash Buffer (+)
Before using the Wash Buffer, DTT is added to the Wash Buffer at the appropriate concentration.

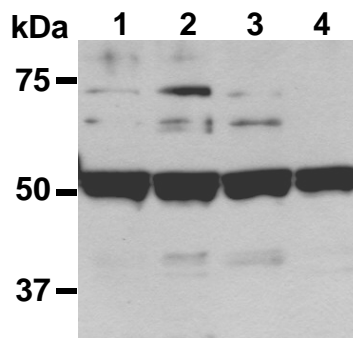
Protocol

- 1) Wash 6×10^6 cells 2 times with PBS and resuspend them with 500 μ L of ice-cold Lysis Buffer (+) containing appropriate protease inhibitors, RNase inhibitors, and DTT. Vortex for 10 seconds. Leave on ice for 10 minutes.
- 2) Centrifuge the tube at 12,000 x g for 5 minutes at 4°C and transfer the supernatant to another tube.
- 3) Add 25 μ L of 50% protein A agarose beads slurry resuspended in Lysis Buffer (+) into the supernatant. Incubate it at 4°C with rotating for 1 hour.
- 4) Centrifuge the tube at 2,000 x g for 1 minute at 4°C and

transfer the supernatant to another tube (precleared sample).

- 5) Mix both 25 μ L of 50% protein A agarose beads slurry resuspended in nuclease-free PBS and Normal Rabbit IgG (RIP-Assay Kit) or anti-PTBP1 antibody at the amount of suggested in the **APPLICATIONS**, and then add 1 mL of Wash buffer (+) into each tube. Incubate with gently agitation for 1 hour at 4°C.
- 6) Wash the beads once with ice-cold Lysis Buffer (+) (centrifuge the tube at 2,000 x g for 1 minute). Carefully discard the supernatant using a pipettor without disturbing the beads.
- 7) Add 500 μ L of cell lysate (precleared sample of step 4), then incubate with gentle agitation for 3 hours at 4°C.
- 8) Wash the beads 4 times with Wash Buffer (+) (centrifuge the tube at 2,000 x g for 1 minute).
- 9) Add 400 μ L of Master mix solution (Solution I: Solution II = 10 μ L: 390 μ L). Vortex for 10 seconds.
- 10) Add 250 μ L of Solution III. Vortex for 10 seconds.
- 11) Centrifuge the tube at 2,000 x g for 2 minutes.
- 12) Transfer the supernatant to the tube containing 2 μ L of Solution IV.
- 13) Add 600 μ L of ice-cold 2-propanol and place at -20°C for 20 minutes. Centrifuge the tube at 12,000 x g for 10 minutes.
- 14) Wash the pellet 2 times with 0.5 mL of ice-cold 70% Ethanol and dry up the pellet for 5-15 minutes.
- 15) Dissolve the pellets in nuclease-free water.
- 16) RNA was quantified with NanoDrop (Thermo Fisher Scientific Inc.) and the RNA quality was analyzed with Bioanalyzer (Agilent Technologies, Inc.).

(Positive control for RNP Immunoprecipitation; Jurkat)



Western blot analysis of PTBP1 expression in K562 (1), 293T (2), HeLa (3) and Jurkat (4) using RN011P.

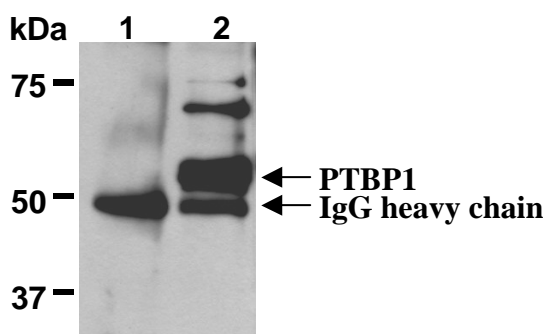
SDS-PAGE & Western Blotting

- 1) Wash 1×10^7 cells 3 times with PBS and suspend them in 1 mL of Laemmli's sample buffer.
- 2) Boil the samples for 2 minutes and centrifuge. Load 10 μ L of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
- 3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine,

20% MeOH). See the manufacturer's manual for precise transfer procedure.

- 4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
- 5) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
- 6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 7) Incubate the membrane with the 1:10,000 HRP-conjugated Anti-rabbit IgG serum (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 8) Wash the membrane with PBS-T (5 minutes x 3 times).
- 9) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 10) Expose to an X-ray film in a dark room for 1 minute. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; K562, 293T, HeLa, Jurkat)



Immunoprecipitation of PTBP1 from Jurkat with normal rabbit IgG (1) or RN011P (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with RN011P.

Immunoprecipitation

- 1) Wash 1×10^7 cells 2 times with PBS and resuspend them with 1 mL of ice-cold Lysis buffer (RIP-Assay Kit) containing appropriate protease inhibitors, then sonicate briefly (up to 10 seconds).
- 2) Centrifuge the tube at 12,000 x g for 5 minutes at 4°C and transfer the supernatant to another tube.
- 3) Add 20 μ L of 50% protein A agarose beads slurry resuspended in Lysis Buffer into the supernatant. Incubate it at 4°C with rotating for 1 hour.
- 4) Centrifuge the tube at 2,000 x g for 1 minute at 4°C and transfer the supernatant to another tube (precleared sample).
- 5) Mix both 20 μ L of 50% protein A agarose beads slurry

resuspended in nuclease-free PBS and Normal Rabbit IgG (RIP-Assay Kit) or anti-PTBP1 antibody at the amount of suggested in the **APPLICATIONS**, and then add 1 mL of Wash buffer into each tube. Incubate with gently agitation for 1 hour at 4°C.

- 6) Wash the beads once with ice-cold Lysis Buffer (centrifuge the tube at 2,000 x g for 1 minute). Carefully discard the supernatant using a pipettor without disturbing the beads.
- 7) Add 250 μ L of cell lysate (precleared sample of step 4), then incubate with gentle agitation for 1 hour at 4°C
- 8) Wash the beads 4 times with Wash Buffer (centrifuge the tube at 2,000 x g for 1 minute).
- 9) Resuspend the beads in 20 μ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 20 μ L/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting.**)

(Positive control for Immunoprecipitation; Jurkat)

RELATED PRODUCTS:

RIP Certified Antibody

RN001P	Anti-EIF4E (polyclonal)
RN002P	Anti-EIF4G1 (polyclonal)
RN003P	Anti-EIF4G2 (polyclonal)
RN004P	Anti-ELAVL1/HuR (polyclonal)
RN005P	Anti-ELAVL2/HuB (polyclonal)
RN006P	Anti-ELAVL3/HuC (polyclonal)
RN007P	Anti-IGF2BP1/IMP1 (polyclonal)
RN008P	Anti-IGF2BP2/IMP2 (polyclonal)
RN009P	Anti-IGF2BP3/IMP3 (polyclonal)
RN010P	Anti-MSI1/Musashi1 (polyclonal)
RN011P	Anti-PTBP1 (polyclonal)
RN012P	Anti-STAU1 (polyclonal)
RN013P	Anti-STAU2 (polyclonal)
RN014P	Anti-TIA1 (polyclonal)
RN015P	Anti-YBX1 (polyclonal)
RN1001	RIP-Assay Kit

Isotype control antibody

M075-3	Mouse IgG1 Isotype control
M076-3	Mouse IgG2a Isotype control
M077-3	Mouse IgG2b Isotype control
M078-3	Mouse IgG3 Isotype control
M080-3	Rat IgG1 Isotype control
M081-3	Rat IgG2a Isotype control
M090-3	Rat IgG2b Isotype control
M082-3	Rat IgG2c Isotype control

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