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

RiboCluster Profiler™ **RIP-Certified Antibody**

Anti-PTBP1 (Human) pAb

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 a.a.

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 x 10^6 cells
Western blotting; 1:1,000 for chemiluminescence detection system
Immunoprecipitation; 5 µg/250 µL of cell extract from 2.5 x 10^6 cells
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested*
*It is reported that this antibody can be used in this application in the reference number 4).
Flow cytometry; Not tested
Crosslinking-immunoprecipitation (CLIP); Not tested*
*It is reported that this antibody can be used in enhanced CLIP in the reference number 2).

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

SPECIES CROSS REACTIVITY:

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

REFERENCES:
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2) Nussbacher, J. K. and Yeo, G. W., *Mol. Cell* **69**, 1005-1016.e7 (2018)
3) Wang, Z. N., *et al.*, *Oncotarget* **8**, 36185-36202 (2017) [RIP]
4) Cui, J. and Placzek, W. J., *Cell Death Differ*. **23**, 1681-1690 (2016)
5) Van Nostrand, E. L., *et al.*, *Nat. Methods*. **13**, 508-514 (2016) [CLIP]
6) Sundararaman, B., *et al.*, *Mol. Cell* **61**, 903-913 (2016) [IC, CLIP]
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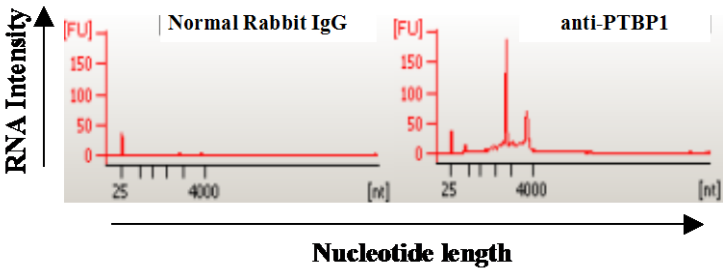
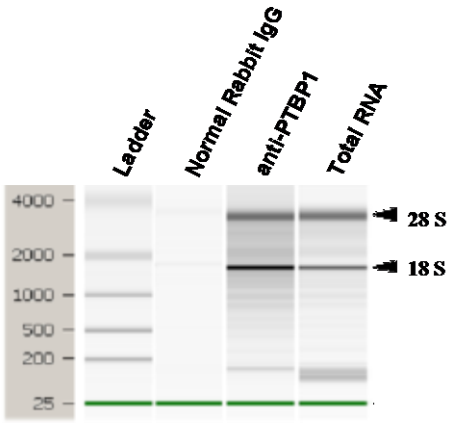
9) Porter, J. F., *et al.*, *J. Immunol*. **181**, 3336-3345 (2008)
10) Pautz, A., *et al.*, *J. Biol. Chem*. **281**, 32294-32302 (2006)
11) Cornelis, S., *et al.*, *Nucleic Acids Res*. **33**, 3095-3108 (2005)

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL http://ruo.mbl.co.jp/je/rip-assay/
e-mail support@mbl.co.jp, TEL 052-238-1904





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 (MBL; code no. RN1001). Please also refer to the protocol packaged in the RIP-Assay Kit.

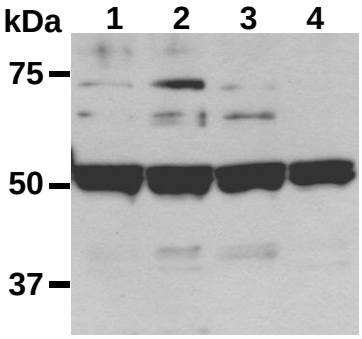
[Material Preparation]

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 x 10^6 cells 2 times with PBS and resuspend them with 500 µL 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 µL 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 µL of 50% protein A agarose beads slurry resuspended in nuclease-free PBS and Normal Rabbit IgG (RIP-Assay Kit) or Anti-PTBP1 (Human) pAb (RN011P) at the amount of suggested in the **APPLICATIONS,** and then add 1 mL of Wash buffer (+) into each tube. Incubate with gentle 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 µL of cell lysate (precleared sample of step 4), then incubate with gentle agitation for 3 hours at 4°C.
8) Centrifuge the tube at 2,000 x g for 1 minute and discard the supernatant.
9) Resuspend the agarose with cold Lysis buffer.
10) Centrifuge the tube at 2,000 x g for 1 minute and discard the supernatant.
11) Repeat steps 8)-10) 4 times.
12) Add 400 µL of Master mix solution (Solution I: Solution II = 10 µL: 390 µL). Vortex for 10 seconds.
13) Add 250 µL of Solution III. Vortex for 10 seconds.
14) Centrifuge the tube at 2,000 x g for 2 minutes.
15) Transfer the supernatant to the tube containing 2 µL of Solution IV.
16) Add 600 µL of ice-cold 2-propanol and place at -20°C for 20 minutes. Centrifuge the tube at 12,000 x g for 10 minutes.
17) Wash the pellet 2 times with 500 µL of ice-cold 70% Ethanol and dry up the pellet for 5-15 minutes.
18) Dissolve the pellets in nuclease-free water.
19) 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)



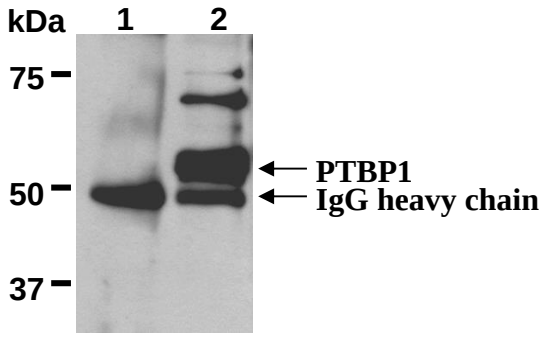
Western blot analysis of PTBP1 expression in K562 (1), 293T (2), HeLa (3) and Jurkat (4) using RN011P.

SDS-PAGE & Western Blotting

1) Wash 1 x 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 µL 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/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture'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 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
7) Incubate the membrane with 1:10,000 of Anti-IgG (Rabbit) pAb-HRP (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 and Jurkat)



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

Immunoprecipitation

1) Wash 1 x 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 µL 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 µL of 50% protein A agarose beads slurry resuspended in nuclease-free PBS and Normal Rabbit IgG (RIP-Assay Kit) or Anti-PTBP1 (Human) pAb (RN011P) at the amount of suggested in the **APPLICATIONS,** and then add 1 mL of Wash buffer into each tube. Incubate with gentle 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 µL of cell lysate (precleared sample of step 4), then incubate with gentle agitation for 1 hour at 4°C.
8) Centrifuge the tube at 2,000 x g for 1 minute and discard the supernatant.
9) Resuspend the agarose with cold Lysis buffer.
10) Centrifuge the tube at 2,000 x g for 1 minute and discard the supernatant.
11) Repeat steps 8)-10) 4 times.
12) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 20 µL/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; Jurkat)

RELATED PRODUCTS:

RIP-Assay Kit

RN1001	RIP-Assay Kit

RIP Certified Antibody

RN019P	Anti-HNRNPK pAb
RN022P	Anti-PABPC4 pAb
RN024P	Anti-PCBP1 pAb
RN025P	Anti-PCBP2 pAb

RBP Antibody

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

RN061PW	Anti-HNRNPA0 pAb
RN114PW	Anti-HNRNPA1 pAb
RN052PW	Anti-HNRNPC pAb
RN060PW	Anti-HNRNPD (AUF1) pAb
RN047PW	Anti-PTBP2 (Human) pAb
RN106PW	Anti-SFPQ (PSF) pAb
RN014MW	Anti-SFPQ (PSF) mAb (C23)
RN084PW	Anti-SRRM1 (SRM160) pAb
RN046PW	Anti-SYNCRIP (HNRNPQ) pAb

For the latest information of RiboCluster Profiler™, Please visit website at http://ruo.mbl.co.jp/je/rip-assay/