

**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<sup>6</sup> cells

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

Immunoprecipitation; 5 µg/250 µL of cell extract from 2.5 x 10<sup>6</sup> 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).

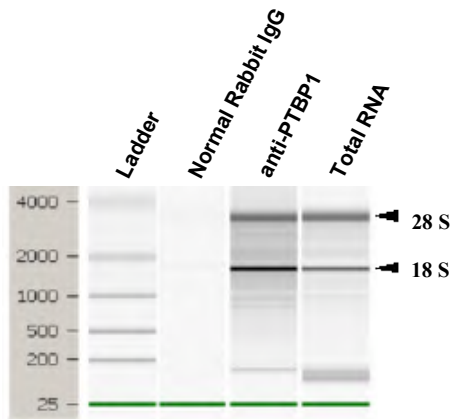
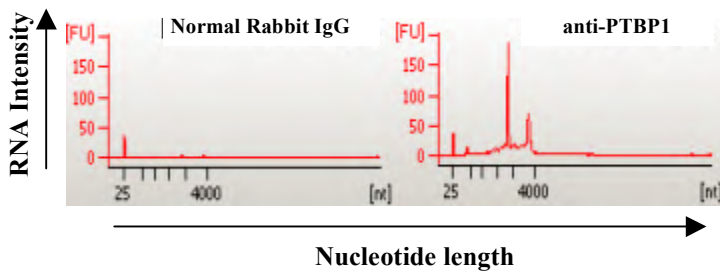
Detailed procedure is provided in the following **PROTOCOLS**.

## SPECIES CROSS REACTIVITY:

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

## REFERENCES:

- 1) Cui, J. and Placzek, W. J., *Cell Death Dis.* **9**, 552 (2018) [WB]
- 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]
- 7) Liu, W., et al., *FASEB J.* **29**, 1113-1123 (2015) [WB]
- 8) Ferrarese, R., et al., *J. Clin. Invest.* **124**, 2861-2874 (2014) [WB, IP, IC, RIP]
- 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)



**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]

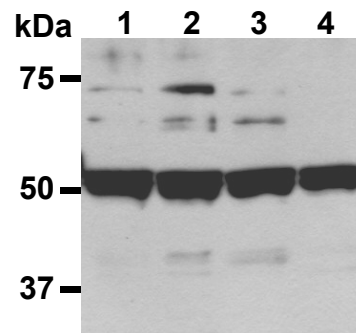
1. Lysis Buffer (+)  
Before using the Lysis Buffer, protease inhibitors, RNase inhibitors, and DTT are added to the Lysis Buffer at the appropriate concentration.
2. Wash Buffer (+)  
Before using the Wash Buffer, DTT is added to the Wash Buffer at the appropriate concentration.

**Protocol**

- 1) Wash  $6 \times 10^6$  cells 2 times with PBS and resuspend them with 500  $\mu$ 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  $\mu$ 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  $\mu$ L 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  $\mu$ L 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  $\mu$ L of Master mix solution (Solution I: Solution II = 10  $\mu$ L: 390  $\mu$ L). Vortex for 10 seconds.
- 13) Add 250  $\mu$ L 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  $\mu$ L of Solution IV.
- 16) Add 600  $\mu$ 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.
- 17) Wash the pellet 2 times with 500  $\mu$ L 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)



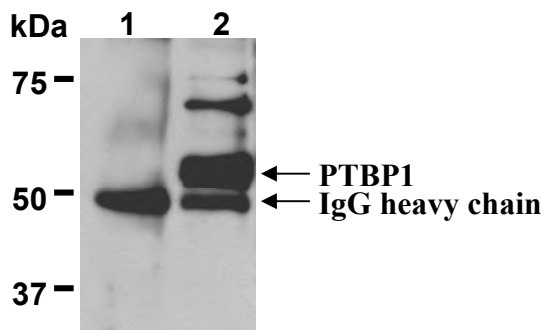
**Western blot analysis of PTBP1 expression in K562 (1), 293T (2), HeLa (3) and Jurkat (4) using RN011P.**

**SDS-PAGE & Western Blotting**

- 1) Wash  $1 \times 10^7$  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  $\mu$ 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/cm<sup>2</sup> 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<sup>7</sup> 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/>