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

RiboCluster Profiler™ RIP-Certified Antibody

Anti-PCBP2 pAb

Code No.	Quantity	Form
RN025P	200 µL	Affinity Purified

BACKGROUND: Poly(rC)-binding proteins (PCBPs) are cellular RNA-binding proteins, characterized by their triple KH structure and by their poly(C)-binding specificity. The 3 KH domains are not only involved in nucleic acid binding but also mediate protein-protein interactions within the RNP complexes. It binds specifically to polypyrimidine tracts within the 3' UTR of target mRNAs through KH domains. PCBPs are expressed as 4 isoforms in cells. PCBP1 and PCBP2 are predominantly expressed in the nucleus and can shuttle from the nucleus to the cytoplasm, while PCBP3 and PCBP4 are expressed to a less extent in the cytoplasm. The most characterized gene regulated by PCBP1 and PCBP2 is the α-globin gene, and the accumulated α-globin mRNA is known to account for approximately 95% of total cellular mRNA during terminal erythroid differentiation. Specific binding of PCBPs to 3′ UTR of mRNA is associated with the stabilization of α-globin mRNA. Microarray analysis of human mRNAs present in a specific subset of PCBP2-RNP complexes also showed a potential involvement of PCBP2 in transcription factor expression via association with their mRNA; further, the enrichment of PCBP2 mRNA in PCBP2-RNP complexes suggested that PCBP2 expression may be subject to posttranscriptional autoregulation.

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, corresponding to internal region of human PCBP2.

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.

REACTIVITY: This antibody reacts with human PCBP2 on Western blotting, Immunoprecipitation and RNP Immunoprecipitation.

INTENDED USE:
For Research Use Only. Not for use in diagnostic procedures.

APPLICATIONS:
RNP Immunoprecipitation; 15 µL/500 µL of cell extract from 1.5 x 10^7 cells
Western blotting; 1:1,000
Immunoprecipitation; 5 µL/500 µL of cell extract from 5 x 10^6 cells
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested*
Flow cytometry; Not tested
Crosslinking-immunoprecipitation (CLIP); Not tested*

*It is reported that this antibody can be used in Immunocytochemistry[1), 3)], enhanced CLIP[2), 3)] and Immunodepletion[5)].

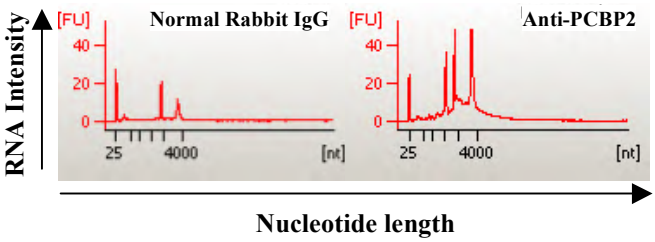
Detailed procedures are provided in the following **PROTOCOLS**.

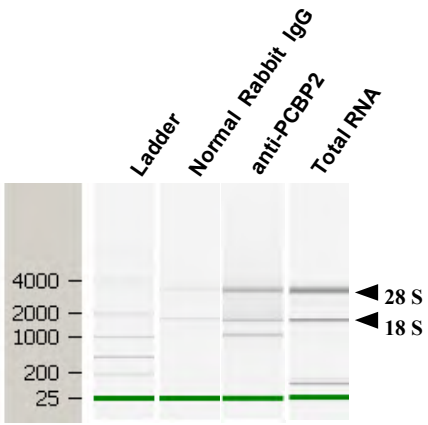
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster
Cells	293T, HeLa, K562, Jurkat, MCF7	NIH/3T3, WR19L	Rat1	Not tested
Reactivity on WB	+	+	+	

REFERENCES:
1) Markmiller, S., *et al., Cell* **172**, 590-604.e13 (2018) [IC]
2) Van Nostrand, E. L., *et al., Nat. Methods* **13**, 508-514 (2016) [CLIP]
3) Sundararaman, B., *et al. Mol. Cell* **61**, 903-913 (2016) [IC, CLIP]
4) Neff, A. T., *et al., Genome Res.* **22**, 1457-1467 (2012) [WB]
5) Palusa, S., *et al., PLoS One* **7**, e33561 (2012) [RIP]
6) Waggoner, S. A., and Liebhaber, S. A., *Mol. Cell. Biol.* **23**, 7055-7067 (2003)

7) Chkheidze, A. N., and Liebhaber, S. A., *Mol. Cell. Biol.* **23**, 8405-8415 (2003)
8) Makeyev, A. V., and Liebhaber, S. A., *RNA* **8**, 265-278 (2002)





Analysis of isolated RNA with Bioanalyzer.

Average of the RNA Quantity (n=2)	
Antibody	RNA (ng)
Normal Rabbit IgG	89.0
anti-PCBP2	369.0
Total RNA	230260.0

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOLS:

RNP Immunoprecipitation

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

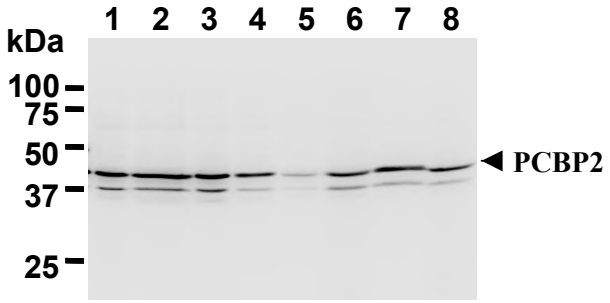
[Material Preparation]

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 1.5 x 10^7 cells 4 times with PBS and resuspend them with 500 µL of ice-cold Lysis Buffer (+) containing appropriate protease inhibitors, RNase inhibitors, and DTT. Vortex thoroughly, then incubate it 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 fresh tube (precleared sample).
5) Mix 25 µL of 50% protein A agarose beads slurry resuspended in nuclease-free PBS with Normal Rabbit IgG (RIP-Assay Kit) or Anti-PCBP2 pAb (RN025P) at the amount as 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) Wash the bead pellet 4 times with Wash Buffer (+) (centrifuge the tube at 2,000 x g for 1 minute).
9) Add 400 µL of Master mix solution (Solution I: Solution II = 10 µL: 390 µL). Vortex thoroughly, then spin-down.
10) Add 250 µL of Solution III. Vortex thoroughly.
11) Centrifuge the tube at 2,000 x g for 2 minutes.
12) Transfer the supernatant to the fresh tube containing 2 µL of Solution IV.
13) 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.
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; K562)



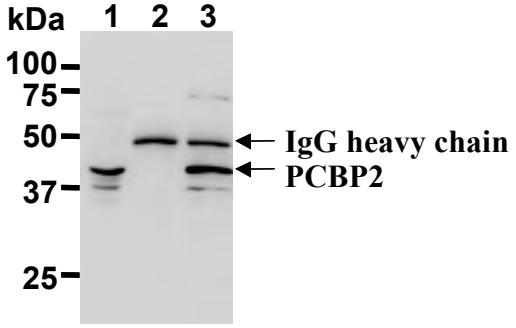
Western blot analysis of PCBP2 expression in 293T (1), HeLa (2), K562 (3), Jurkat (4), MCF7 (5), NIH/3T3 (6) WR19L (7) and Rat1 (8) using RN025P.

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 sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out 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 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 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 off the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute.
10) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
11) The detection was performed with LAS-4000 (FUJIFILM).

(Positive controls for Western blotting; 293T, HeLa, K562, Jurkat, MCF7, NIH3T3, WR19L and Rat1)



Immunoprecipitation of PCBP2 from K562 with normal rabbit IgG (2) or RN025P (3). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with RN025P. Lane 1 is the input sample.*

Immunoprecipitation

1) Wash cells (approximately 1 x 10^7 cells) 2 times with PBS and resuspend them with 1 mL of ice-cold Lysis buffer (RIP-Assay Kit) containing protease inhibitors and DTT at appropriate concentrations. 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 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 20 µL of 50% protein A agarose beads slurry resuspended in PBS with Normal Rabbit IgG (RIP-Assay Kit) or Anti-PCBP2 pAb (RN025P) at the amount as 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) 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 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; K562)

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