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

RiboCluster Profiler™

RIP-Certified Antibody

Anti-PCBP1 pAb

Code No.	Quantity	Form
RN024P	200 µL	Affinity Purified

BACKGROUND: Poly(rC)-binding proteins (PCBPs) are cellular RNA-binding proteins; these proteins are defined by their triple KH structure and poly(C)-binding specificity. The 3 KH domains are not only involved in nucleic acid binding but also in mediating protein-protein interactions within the RNP complexes. PCBPs bind specifically to polypyrimidine tracts within the 3′ UTR of target mRNAs through the KH domains. PCBPs are expressed as 4 isoforms in cells. PCBP1 and PCBP2 are widely expressed in both the nucleus and the cytoplasm and can shuttle from the nucleus to the cytoplasm, while PCBP3 and PCBP4 are expressed to a less extent. PCBP1 and PCBP2 have been reported to stabilize the target mRNAs such as those of α-globin, α1(I) collagen, and tyrosine hydroxylase by binding to C-rich 3′ UTR motifs. The 15-lipoxygenase mRNA is translationally silent until reticulocytes in the peripheral blood undergo the final steps of maturation. This translational silencing has been shown to be mediated by the binding of PCBP1 and hnRNPK to the 3′ UTR of 15-lipoxygenase mRNA.

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 PCBP1.

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 PCBP1 on Western blotting, Immunoprecipitation and RNP Immunoprecipitation.

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
Other; Not tested*

*It is reported that this antibody can be used in Immunocytochemistry[3] and EMSA[2].

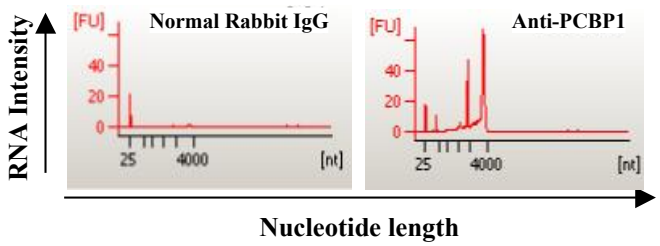
Detailed procedures are provided in the following **PROTOCOLS**.

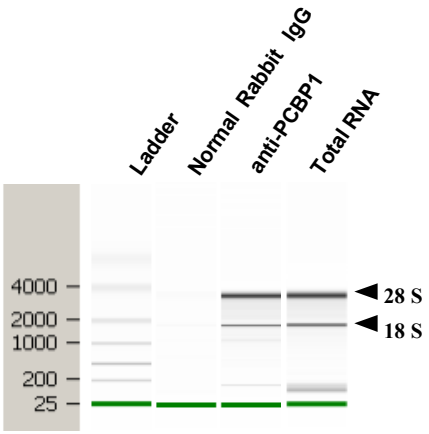
SPECIES CROSS REACTIVITY:

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

REFERENCES:
1) Espinoza-Lewis, R. A., *et al., J. Biol. Chem.* **292**, 9540-9550 (2017) [WB]
2) Yabe-Wada, T., *et al., Sci. Rep.* **6**, 26566 (2016) [RIP, EMSA]
3) Sundararaman, B., *et al.*, *Mol. Cell* **61**, 903-913 (2016) [IC]
4) Makeyev, A. V., and Liebhaber, S. A., *RNA* **8**, 265-278 (2002)
5) Ostareck, D. H., *et al., Cell* **89**, 597-606 (1997)

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





Analysis of isolated RNA with Bioanalyzer.

Average of the RNA Quantity (n=2)	
Antibody	RNA (ng)
Normal Rabbit IgG	54.5
anti-PCBP1	1580.0
Total RNA	243590.0

PROTOCOLS:

RNP Immunoprecipitation

Some buffers 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.

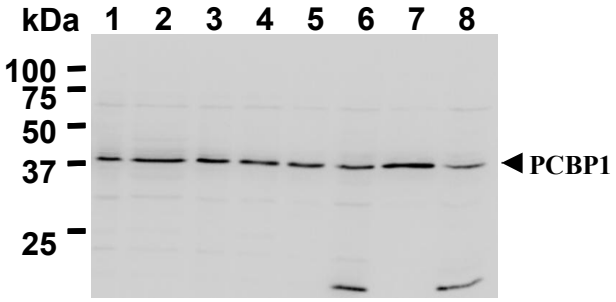
[Precaution]

RNP Immunoprecipitation using this antibody requires the addition of 30 µL of High-Salt Solution (RIP-Assay Kit) to each mL of Lysis Buffer (+) and Wash Buffer (+) just before use.

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-PCBP1 pAb (RN024P) at the concentration 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)



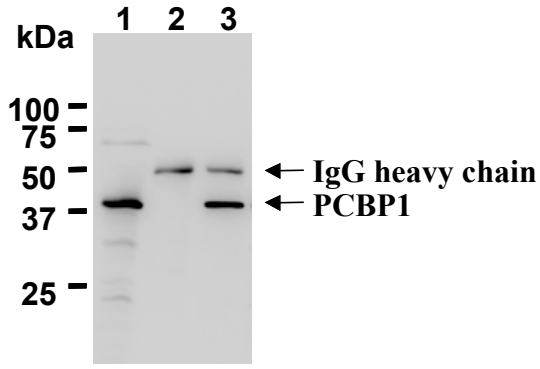
Western blot analysis of PCBP1 expression in 293T (1), HeLa (2), K562 (3), Jurkat (4), MCF7 (5), NIH/3T3 (6), WR19L (7) and Rat1 (8) using RN024P.

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 PCBP1 from K562 with normal rabbit IgG (2) or RN024P (3). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with RN024P. Lane 1 is the input sample.*

RBP Antibody

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

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 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.
2) 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.
3) Centrifuge the tube at 2,000 x g for 1 minute at 4°C and transfer the supernatant to another tube (precleared sample).
4) Mix 20 µL of 50% protein A agarose beads slurry resuspended in PBS with Normal Rabbit IgG (RIP-Assay Kit) or Anti-PCBP1 pAb (RN024P) at the concentration 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.
5) 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.
6) Add 500 µL of cell lysate (precleared sample of step 4), then incubate with gentle agitation for 3 hours at 4°C.
7) Wash the beads 4 times with Wash Buffer (centrifuge the tube at 2,000 x g for 1 minute).
8) Resuspend the bead pellet 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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