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

RiboCluster Profiler™

RIP-Certified Antibody

Anti-CIRBP

Code No.	Quantity	Concentration	Form
RN032P	200 µL	1 mg/mL	Affinity Purified

BACKGROUND: The cold-inducible RNA-binding protein (CIRBP) contains an N-terminal RNA binding domain and a C-terminal Gly-rich domain. It exhibits specific RNA-binding activity owing to its ability to bind to poly (U) even at high NaCl concentrations. CIRBP transcripts are expressed in cells such as K562, HepG2, HeLa, and T24. In all of them, the transcript and translation levels of CIRBP were increased when the culture temperature was lowered from 37 to 32 ^{o}C. CIRBP affects gene expression by facilitating translation under mild cold stress, and it may play an essential role in cold-induced suppression of cell proliferation.

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 N-terminus of human CIRBP.

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^{o}C.

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

APPLICATIONS:
RNP Immunoprecipitation; 15 µg/500 µL of cell extract from 2 x 10^{7} cells
Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; 5 µg/500 µL of cell extract from 5 x 10^{6} cells
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

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

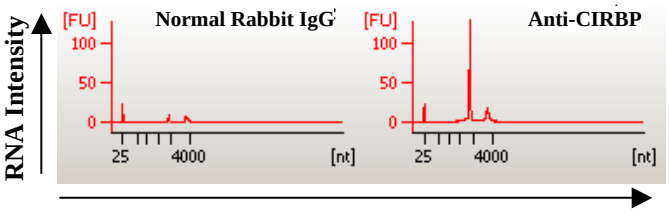
REFERENCES:
1) Artero-Castro, A., *et al.*, *Mol. Cell Biol.* **29,** 1855-1868 (2009)
2) Nishiyama, H., *et al.*, *Gene* **204,** 115-120 (1997)
3) Nishiyama, H., *et al.*, *J. Cell Biol.* **137,** 899-908 (1997)

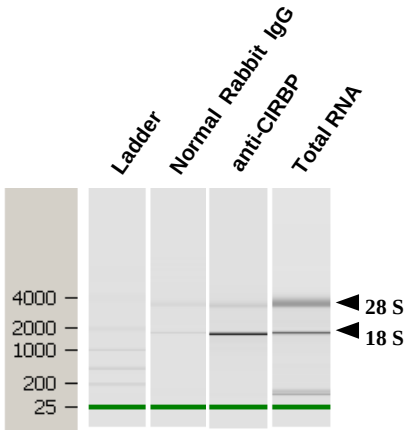
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster
Cells	293T, HeLa, K562, Jurkat	NIH/3T3	Rat1	Not Tested
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@mbl.co.jp

MBL MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL https://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	87.0
anti-CIRBP	1014.5
Total RNA	388385.0

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.

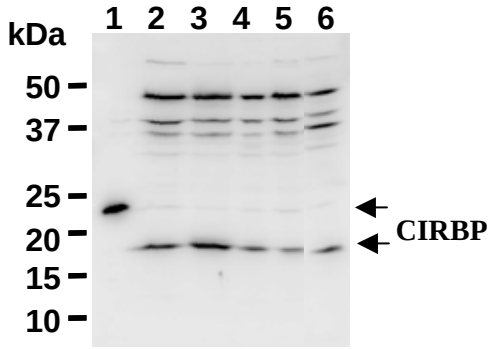
[Material Preparation]

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 2 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-CIRBP antibody at the concentration 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 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) 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; Jurkat)



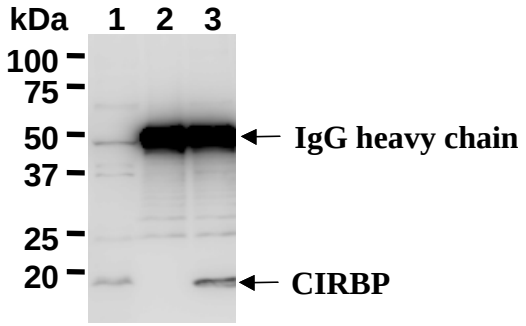
Western blot analysis of CIRBP expression in transfectant (1), HeLa (2), 293T (3), K562 (4), Jurkat (5) and Rat1 (6) using RN032P.

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 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 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 the 1:10,000 HRP-conjugated anti-rabbit IgG (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, NIH3T3, WR19L, Rat1)



Immunoprecipitation of CIRBP from HeLa with normal rabbit IgG (2) or RN032P (3). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with RN032P. 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 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 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-CIRBP antibody at the concentration 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 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; HeLa)

RELATED PRODUCTS:

RIP-Assay Kit

RN1001	RIP-Assay Kit

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)

Other RIP-Certified Antibodies are also available.
Please visit our website at
https://ruo.mbl.co.jp/product/epigenetics/rip-assay.html

RIP-Assay Starter Kit

Each RIP-Assay Starter Kit contains 40 µg of RIP-Certified Antibody and RIP-Assay Kit.

RN001PK	RIP-Assay Starter Kit EIF4E (polyclonal)
RN002PK	RIP-Assay Starter Kit EIF4G1 (polyclonal)
RN003PK	RIP-Assay Starter Kit EIF4G2 (polyclonal)
RN004PK	RIP-Assay Starter Kit ELAVL1/HuR (polyclonal)
RN005PK	RIP-Assay Starter Kit ELAVL2/HuB (polyclonal)
RN006PK	RIP-Assay Starter Kit ELAVL3/HuC (polyclonal)
RN007PK	RIP-Assay Starter Kit IGF2BP1/IMP1 (polyclonal)
RN008PK	RIP-Assay Starter Kit IGF2BP2/IMP2 (polyclonal)
RN009PK	RIP-Assay Starter Kit IGF2BP3/IMP3 (polyclonal)
RN010PK	RIP-Assay Starter Kit MSI1/Musashi1 (polyclonal)

Other RIP Starter Kits are also available.
Please visit our website at
https://ruo.mbl.co.jp/product/epigenetics/rip-assay.html

RBP Antibody

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

RN023PW	Anti-PABPN1 (polyclonal)
RN028PW	Anti-EIF2C1/AGO1 (polyclonal)
RN029PW	Anti-EIF2C2/AGO2 (polyclonal)
RN030PW	Anti-DICER1 (polyclonal)
RN031PW	Anti-ZFP36 (polyclonal)
RN034PW	Anti-CUGBP1 (polyclonal)
RN035PW	Anti-CUGBP2 (polyclonal)