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

MBL

RiboCluster Profiler™ **RIP-Certified Antibody**

Anti-IGF2BP1 (IMP1) mAb

Code No.	Clone	Subclass	Quantity	Concentration
RN001M	6H6	Mouse IgG2a κ	200 µL	1 mg/mL

BACKGROUND: Localization of IGF2 mRNA-binding protein 1 (IMP1) is associated with motility, and the major functions of IMP1 are carried out by the phylogenetically conserved K homology (KH) domains. IMP1 can affect stability, localization, and translation of its target RNAs. Binding of IMP1 to the leader 3 mRNA in the 5'-untranslated region (UTR) of IGF2 inhibits translation from a leader 3 reporter mRNA. Aberrant IMP1 expression may interfere with c-myc regulation. In tumors, gains at 8q24 (c-myc locus) were observed to be more frequent than those at 17q21 (IMP1 locus). Furthermore, IMP1 expression was frequently detected in tumors, implying that the mechanism of activation is other than that of gene amplification.

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 hybridoma (clone 6H6) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with C3H mouse lymphocyte immunized with full length of human IGF2BP1.

FORMULATION: 200 µg IgG in 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 IGF2BP1 (~63 kDa) on Western blotting, Immunoprecipitation and RNP Immunoprecipitation.

INTENDED USE:
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APPLICATIONS:
RNP Immunoprecipitation; 15 µg/500 µL of cell extract from 1 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**.

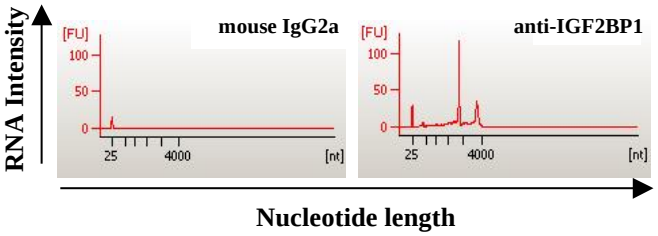
REFERENCES:
1) Ioannidis, P., *et al., Int. J. Cancer* **104**, 54-59 (2003)
2) Nielsen, F. C., *et al., J. Cell Sci.* **115**, 2087-2097 (2002)
3) Nielsen, J., *et al., Mol. Cell Biol.* **19**, 1262-1270 (1999)

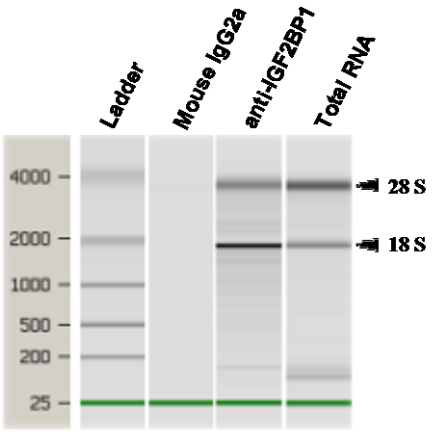
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster
Cells	K562, 293T, HeLa, HL-60	NIH/3T3, MEF	Not tested	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 the purposes of their own research. Researchers are not allowed to use this product or RIP-Assay technology for commercial purposes without acquiring a license. For commercial use and licensing opportunities, please contact us at RIP@mbl.co.jp

MBL 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)
mouse IgG2a	65.0
anti-IGF2BP1 mAb	614.0
Total RNA	181500.0

PROTOCOLS:

RNP Immunoprecipitation

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

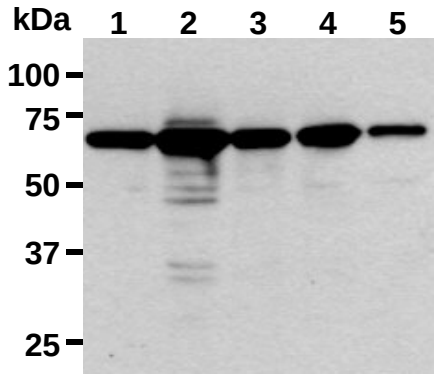
[Material Preparation]

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 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 tube (precleared sample).
5) Mix 25 µL of 50% protein A agarose beads slurry resuspended in nuclease-free PBS with Mouse IgG2a (isotype control) (MBL; code no. M076-3) or Anti-IGF2BP1 (IMP1) mAb (RN001M) at the concentration suggested in **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 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 let the pellet dry for 5-15 minutes.
15) Dissolve the pellets in nuclease-free water.
16) RNA was quantified using NanoDrop (Thermo Fisher Scientific Inc.) and the RNA quality was determined using the Bioanalyzer (Agilent Technologies, Inc.).

(Positive control for RNP Immunoprecipitation; K562)



Western blot analysis of IGF2BP1 expression in HeLa (1), 293T (2), HL-60 (3), NIH/3T3 (4) and MEF (5) using RN001M.

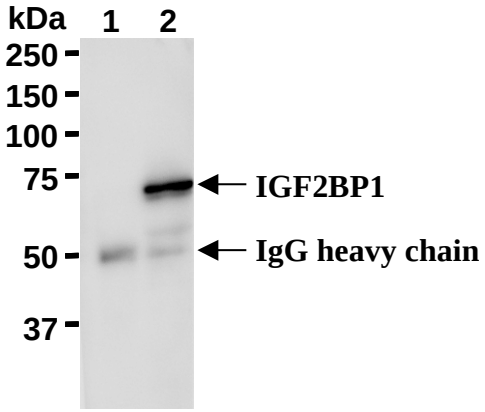
SDS-PAGE & Western Blotting

1) Wash cells (approximately 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 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 for 1 hour at room temperature with primary antibody diluted with PBS (pH 7.2) containing 1% skimmed milk as suggested in the **APPLICATIONS**. (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 Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) 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; HeLa, 293T, HL-60, NIH/3T3, MEF)



Immunoprecipitation of IGF2BP1 from K562 with mouse IgG2a (1) or RN001M (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with RN001M.*

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 (MBL; code. RN1001) 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 fresh 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 fresh tube (precleared sample).
5) Mix 20 µL of 50% protein A agarose beads slurry resuspended in PBS with Mouse IgG2a (isotype control) (MBL; code no. M076-3) or Anti-IGF2BP1 (IMP1) mAb (RN001M) at the concentration suggested in **APPLICATIONS,** and then add 1 mL of Wash buffer (MBL; code. RN1001) 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)

RELATED PRODUCTS:

RIP-Assay Kit

RN1001	RIP-Assay Kit
RN1005	RIP-Assay Kit *for microRNA*

RIP-Certified Antibody

RN001M	Anti-IGF2BP1 (IMP1) mAb (6H6)
RN003M	Anti-EIF2C2 (AGO2) mAb (1B1-E2H5)
RN004M	Anti-Ribosomal P0/P1/P2 mAb (9D5)
RN005M	Anti-EIF2C2 (AGO2) mAb (2A8)
RN006M	Anti-EIF4E mAb (C107-3-5)
RN007M	Anti-ELAVL1 (HuR) mAb (C67-1)
RN009M	Anti-PABPC1 mAb (10E10)
RN001P	Anti-EIF4E pAb
RN002P	Anti-EIF4G1 pAb
RN003P	Anti-EIF4G2 pAb
RN004P	Anti-ELAVL1 (HuR) pAb
RN005P	Anti-ELAVL2 (HuB) pAb
RN006P	Anti-ELAVL3 (HuC) pAb
RN007P	Anti-IGF2BP1 (IMP1) pAb
RN008P	Anti-IGF2BP2 (IMP2) pAb
RN009P	Anti-IGF2BP3 (IMP3) pAb
RN010P	Anti-MSI1 (Musashi1) pAb
RN011P	Anti-PTBP1 pAb
RN012P	Anti-STAU1 pAb
RN013P	Anti-STAU2 pAb
RN014P	Anti-TIA1 pAb
RN015P	Anti-YBX1 pAb
RN016P	Anti-FMR1 pAb
RN017P	Anti-FXR1 pAb
RN018P	Anti-FXR2 pAb

RN019P Anti-HNRNPK pAb
RN020P Anti-ILF3 pAb
RN021P Anti-KHDRBS1 pAb
RN022P Anti-PABPC4 pAb
RN024P Anti-PCBP1 pAb
RN025P Anti-PCBP2 pAb
RN026P Anti-PUM1 pAb
RN027P Anti-PUM2 pAb
RN028P Anti-EIF2C1 (AGO1) pAb
RN032P Anti-CIRBP pAb
RN033P Anti-TNRC6A (GW182) pAb
RN037P Anti-AUH pAb
RN038P Anti-CPEB1 pAb
RN041P Anti-KHDRBS2 (SLM1) pAb
RN045P Anti-SLBP pAb

RBP Antibody

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

RN002MW Anti-CUGBP1 mAb (3B1)
RN008MW Anti-ELAVL1 (HuR) mAb (C54-6)
RN010MW Anti-PIWIL1 (MIWI) mAb (2D9)

RN023PW Anti-PABPN1 pAb
RN028PW Anti-EIF2C1 (AGO1) pAb
RN029PW Anti-EIF2C2 (AGO2) pAb
RN030PW Anti-DICER1 pAb
RN031PW Anti-ZFP36 (TTP) pAb
RN034PW Anti-CUGBP1 pAb
RN035PW Anti-CUGBP2 pAb
RN036PW Anti-ACO1 (IRP1) pAb
RN039PW Anti-CPEB2 pAb
RN040PW Anti-CPEB4 pAb
RN042PW Anti-MBNL1 pAb
RN043PW Anti-NOVA1 pAb
RN044PW Anti-NOVA2 pAb
RN046PW Anti-SYNCRIP (HNRNPQ) pAb
RN047PW Anti-PTBP2 pAb
RN048PW Anti-G3BP1 pAb
RN049PW Anti-G3BP2 pAb
RN050PW Anti-GRSF1 pAb
RN051PW Anti-HDLBP (Vigilin) pAb
RN052PW Anti-HNRNPC pAb
RN053PW Anti-PAIP1 pAb
RN054PW Anti-PCBP3 pAb
RN055PW Anti-AIMP1 (SCYE1) pAb
RN056PW Anti-SERBP1 pAb
RN057PW Anti-TARBP1 pAb
RN058PW Anti-TARBP2 pAb
RN059PW Anti-TIAL1 pAb
RN060PW Anti-HNRNPD (AUF1) pAb
RN061PW Anti-HNRNPA0 pAb
RN062PW Anti-DGCR8 pAb
RN063PW Anti-DHX9 pAb
RN064PW Anti-FUSIP1 (SRSF10) pAb
RN065PW Anti-KHSRP pAb
RN066PW Anti-KIAA0020 pAb
RN067PW Anti-PPP1R10 pAb
RN068PW Anti-PPP1R8 pAb
RN069PW Anti-RBM14 pAb
RN070PW Anti-RPS10 pAb
RN071PW Anti-RPS19 pAb
RN072PW Anti-RPS6 pAb
RN073PW Anti-RPS9 pAb
RN074PW Anti-SSB (La) pAb
RN075PW Anti-PPARGC1B pAb
RN076PW Anti-PPRC1 pAb
RN077PW Anti-SMN1 pAb
RN078PW Anti-SMNDC1 pAb
RN079PW Anti-SRSF7 (9G8) pAb
RN080PW Anti-SRSF3 (SRp20) pAb
RN081PW Anti-SRSF9 (SRp30c) pAb
RN082PW Anti-SRSF5 (SRP40) pAb
RN083PW Anti-AQR (IBP160) pAb
RN084PW Anti-SRRM1 (SRM160) pAb
RN085PW Anti-U2AF1 pAb
RN086PW Anti-U2AF2 pAb
RN087PW Anti-ALYREF (THOC4) pAb
RN088PW Anti-NXF1 (TAP) pAb
RN089PW Anti-MAGOH pAb
RN090PW Anti-DDX21 pAb
RN091PW Anti-DDX23 pAb
RN092PW Anti-NONO (P54NRB) pAb
RN093PW Anti-PRPF4 pAb
RN094PW Anti-PRPF8 pAb
RN095PW Anti-SNRNP200 pAb
RN096PW Anti-SNRNP40 pAb
RN097PW Anti-SNRNP70 pAb
RN098PW Anti-EDC4 pAb
RN099PW Anti-EIF4A1 pAb
RN100PW Anti-EXOSC5 (RRP46) (Human) pAb
RN101PW Anti-FBL (Fibrillarin) pAb
RN102PW Anti-GEMIN2 (Human) pAb
RN103PW Anti-NCBP1 (CBP80) pAb
RN104PW Anti-PAN2 (USP52) (Human) pAb
RN105PW Anti-PARN pAb
RN106PW Anti-SFPQ (PSF) pAb
RN107PW Anti-TARDBP (TDP-43) pAb
RN108PW Anti-UPF1 pAb
RN109PW Anti-XRN1 (Human) pAb

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

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