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

MBL
A JSR Life Sciences Company

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 recombinant 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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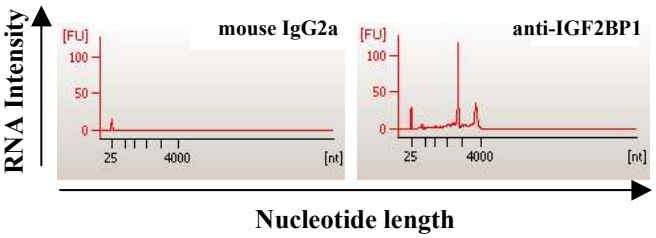
**APPLICATIONS:**
RNP Immunoprecipitation; 15 µg/500 µL of cell extract from 1 x $10^7$ cells
Western blotting; 1 µg/mL
Immunoprecipitation; 5 µg/500 µL of cell extract from 5 x $10^6$ cells
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

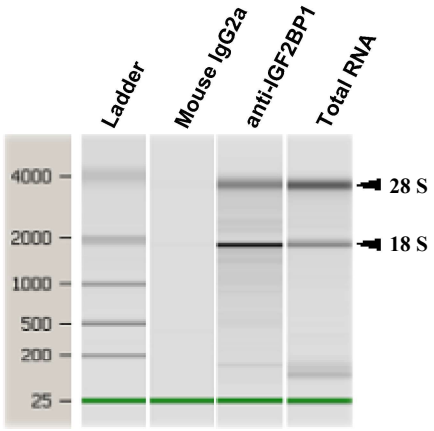
Detailed procedures are provided in the following **PROTOCOLS**.

**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 | + | + | | |




***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 |

The descriptions of the following protocols are examples.

Each user should determine the appropriate condition.

## PROTOCOLS:

### RNP Immunoprecipitation

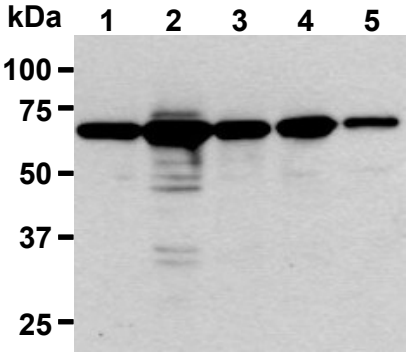
Some buffers and reagents are included in the RIP-Assay Kit (MBL; code. RN1001). Please also refer to the protocol packaged in the RIP-Assay Kit.

[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 twice 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)



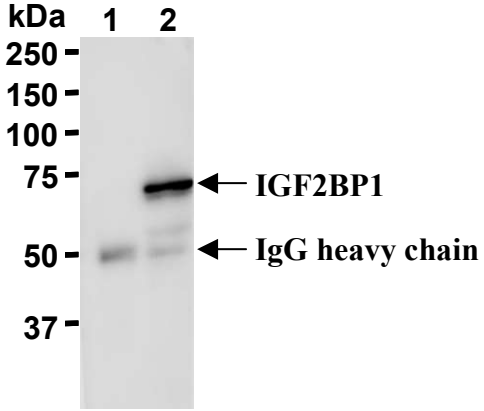

***Western blotting analysis of IGF2BP1 expression in HeLa (1), 293T (2), HL-60 (3), NIH/3T3 (4) and MEF (5) using RN001M.***

### 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% Methanol]). 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).
7) Incubate the membrane with 1:10,000 Anti-IgG (H+L chain) (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).
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) twice 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)

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