

**RiboCluster Profiler™**

**RIP-Certified Antibody**

# Anti-MSI1/Musashi1

Code No.	Quantity	Concentration	Form
RN010P	200 µL	1 mg/mL	Affinity Purified

**BACKGROUND:** Musashi1 (MSI1) is a highly conserved RNA binding protein with important functions in stem cell maintenance, nervous system development and tumorigenesis. MSI1 inhibit translation initiation of its target mRNA, *m-Numb*, by competing with eIF4G for Poly A Binding Protein. MSI1 is mainly expressed in stem and progenitor cells from different tissues in mammals, and a decrease occurs as cells commit to lineage differentiation. Recently, expression of MSI1 in mature photoreceptors and retinal pigment epithelium cells was found and its expression in those cells are absolutely necessary for survival of photoreceptors. Abreu and his coworkers performed a RIP-Chip analysis and revealed that MSI1 associated mRNAs are belong to two main functional categories pertinent to tumorigenesis.

## 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, METDAPQGLASPDSPHDPC corresponding to 1-20 aa.

**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 MSI1 (~39 kDa) on Western blotting, Immunoprecipitation and RNP Immunoprecipitation. Its ability to immunoprecipitate mRNP complex was confirmed by using transfectant.

## APPLICATIONS:

RNP Immunoprecipitation: 15 µg/500 µL of cell extract from  $1.5 \times 10^7$  cells

Western blotting: 1:1,000 for chemiluminescence detection system

Immunoprecipitation: 5 µg/250 µL of cell extract from  $2.5 \times 10^6$  cells

Immunohistochemistry: 1:500

Heat treatment is necessary for paraffin embedded sections. Microwave oven; 2 times for 10 minutes each in 10 mM citrate buffer (pH 6.3)

Immunocytochemistry: Not tested

Flow cytometry: Not tested

Detailed procedure is provided in the following **PROTOCOLS.**

## REFERENCES:

- 1) Abreu, R. S., *et al.*, *J. Biol. Chem.* E publication March 3, (2009)
- 2) Susaki, K., *et al.*, *Exp. Eye Res.* **88**, 347-355 (2009)
- 3) Kawahara, H., *et al.*, *J. Cell Biol.* **181**, 639-653 (2008)
- 4) Imai, T., *et al.*, *Mol. Cell Biol.* **21**, 3888-38900 (2001)

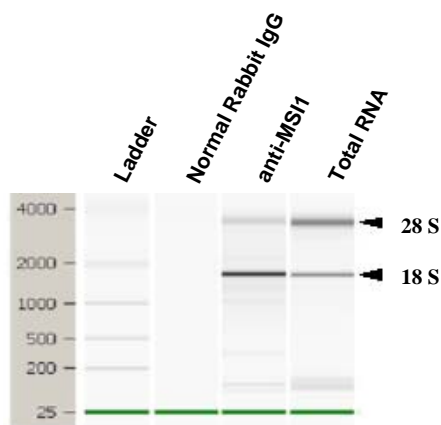
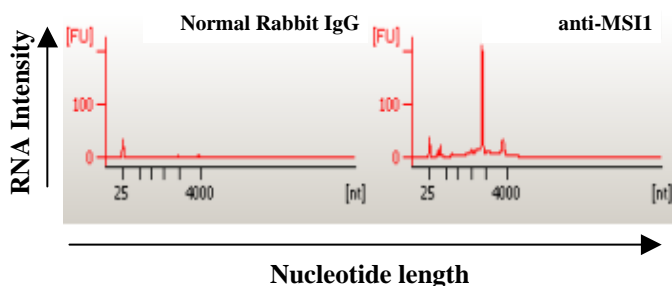
## SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster
Cells and Tissue	SK-N-SH, U251, GOTO, IMR-32, SK-SY-5Y	brain, P19 (RA+)	PC12 (NGF+)	Not Tested
Reactivity on WB	+	+	+	

RA: Retinoic Acid

NGF: Nerve Growth Factor

**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](mailto:RIP@mbl.co.jp)



### Analysis of isolated RNA with Bioanalyzer.

Average of the RNA Quantity (n=2)	
Antibody	RNA (ng)
Normal Rabbit IgG	33.0
anti-MSI1	1178.0
Total RNA	193570.0

## PROTOCOLS:

### RNP Immunoprecipitation

Some buffer 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]

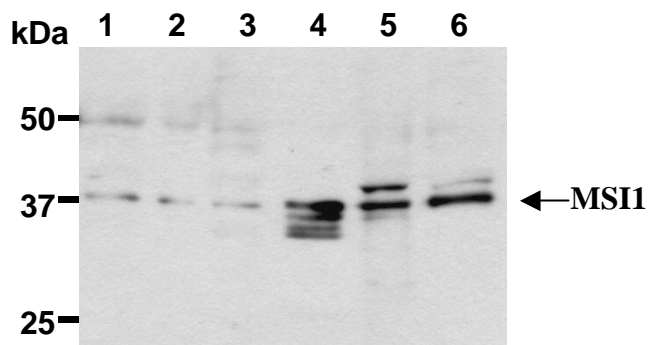
1. Lysis Buffer (+)  
Before using the Lysis Buffer, protease inhibitors, RNase inhibitors, and DTT are added to the Lysis Buffer at the appropriate concentration.
2. Wash Buffer (+)  
Before using the Wash Buffer, DTT is added to the Wash Buffer at the appropriate concentration.

#### Protocol

- 1) Wash  $1.5 \times 10^7$  cells 2 times with PBS and resuspend them with 500  $\mu$ L of ice-cold Lysis Buffer (+) containing appropriate protease inhibitors, RNase inhibitors, and DTT. 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 25  $\mu$ L 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 both 25  $\mu$ L of 50% protein A agarose beads slurry resuspended in nuclease-free PBS and Normal Rabbit IgG (RIP-Assay Kit) or anti-MSI1 antibody at the amount of 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  $\mu$ L 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  $\mu$ L of Master mix solution (Solution I: Solution II = 10  $\mu$ L: 390  $\mu$ L). Vortex for 10 seconds.
- 10) Add 250  $\mu$ L of Solution III. Vortex for 10 seconds.
- 11) Centrifuge the tube at 2,000 x g for 2 minutes.
- 12) Transfer the supernatant to the tube containing 2  $\mu$ L of Solution IV.
- 13) Add 600  $\mu$ L 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; transfectant)



### **Western blot analysis of MSI1 expression in SK-N-SH (1), IMR-32 (2), U251 (3), P19 (RA+) (4), PC12 (NGF+) (5) and SK-SY-5Y (6) using RN010P.**

RA: Retinoic Acid, NGF: Nerve Growth Factor

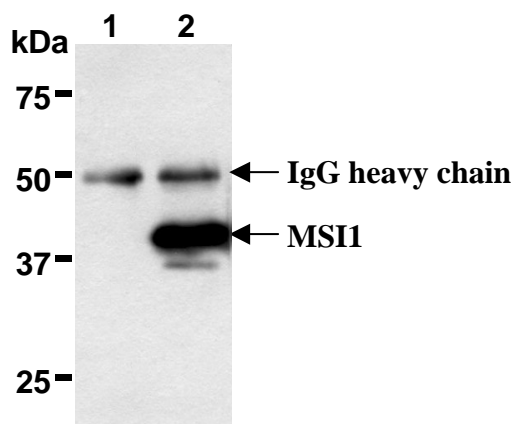
### SDS-PAGE & Western Blotting

- 1) Wash  $1 \times 10^7$  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  $\mu$ L of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
- 3) Blot the protein to a polyvinylidene difluoride (PVDF)

membrane at 1 mA/cm<sup>2</sup> 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 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 condition.)
- 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 serum (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 on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 10) Expose to an X-ray film in a dark room for 1 minute. Develop the film as usual. The condition for exposure and development may vary.

[Positive controls for Western blotting; SK-N-SH, IMR-32, U251, P19 (RA+), PC12 (NGF+), SK-SY-5Y]



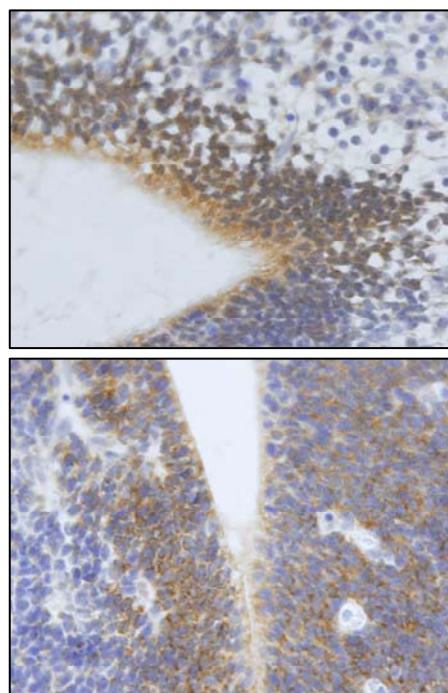
**Immunoprecipitation of MSI1 from transfectant with normal rabbit IgG (1) or RN010P (2).** After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with RN010P.

#### Immunoprecipitation

- 1) Wash  $1 \times 10^7$  cells 2 times with PBS and resuspend them with 1 mL of ice-cold Lysis buffer (RIP-Assay Kit) containing appropriate protease inhibitors, then sonicate briefly (up to 10 seconds).
- 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  $\mu$ L 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 both 20  $\mu$ L of 50% protein A agarose beads slurry resuspended in nuclease-free PBS and Normal Rabbit IgG (RIP-Assay Kit) or anti-MSI1 antibody at the amount of 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 250  $\mu$ L of cell lysate (precleared sample of step 4), then incubate with gentle agitation for 1 hour 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  $\mu$ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 20  $\mu$ L/lane for the SDS-PAGE analysis.  
(See **SDS-PAGE & Western blotting.**)



**Immunohistochemical detection of MSI1 on paraffin embedded section of mouse whole embryo at E14.5 with RN010P.**  
upper panels: ventricular zone  
lower panels: pallidum and septum

#### Immunohistochemical staining for paraffin-embedded sections

- 1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
- 2) Wash the slides with Ethanol 3 times for 3-5 minutes each.

- 3) Wash the slides with PBS 3 times for 3-5 minutes each.
- 4) Heat treatment  
Heat treatment by Microwave:  
Place the slides put on staining basket in 500 mL beaker with 500 mL of 10 mM citrate buffer (pH 6.5). Cover the beaker with plastic wrap, then process the slides 2 times for 10 minutes each at 500 W with microwave oven. Let the slides cool down in the beaker at room temperature for about 40 minutes.
- 5) Remove the slides from the citrate buffer and cover each section with 3% H<sub>2</sub>O<sub>2</sub> in PBS for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
- 6) Remove the slides from PBS, wipe gently around each section and cover tissues with Blocking buffer (20 mM HEPES, 1% BSA, 135 mM NaCl) for 5 minutes to block non-specific staining. Do not wash.
- 7) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with blocking bufer as suggest in the **APPLICATIONS**.
- 8) Incubate the sections for 1 hour at room temperature.
- 9) Wash the slides 3 times in PBS for 5 minutes each.
- 10) Wipe gently around each section and cover tissues with ENVISION+ Dual Link (Dako; code no. K4063). Incubate for 30 minutes at room temperature. Wash as in step 9).
- 11) Visualize by reacting for 10-20 minutes with DAB substrate kit (Dako; code no. K3466). \*DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
- 12) Wash the slides in water for 5 minutes.
- 13) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
- 14) Now ready for mounting.

(Positive controls for Immunohistochemistry; mouse embryonic ventricular zone, pallidum and septum)

Isotype control antibody

M075-3	Mouse IgG1 Isotype control
M076-3	Mouse IgG2a Isotype control
M077-3	Mouse IgG2b Isotype control
M078-3	Mouse IgG3 Isotype control
M080-3	Rat IgG1 Isotype control
M081-3	Rat IgG2a Isotype control
M090-3	Rat IgG2b Isotype control
M082-3	Rat IgG2c Isotype control

**RELATED PRODUCTS:**

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/Musashi 1 (polyclonal)
RN011P	Anti-PTBP1 (polyclonal)
RN012P	Anti-STAU1 (polyclonal)
RN013P	Anti-STAU2 (polyclonal)
RN014P	Anti-TIA1 (polyclonal)
RN015P	Anti-YBX1 (polyclonal)
RN1001	RIP-Assay Kit

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