

**RiboCluster Profiler™**

RIP-Certified Antibody

# Anti-Ribosomal P0/P1/P2 mAb

<b>CODE No.</b>	RN004M
<b>CLONALITY</b>	Monoclonal
<b>CLONE</b>	9D5
<b>ISOTYPE</b>	Mouse IgG2a $\kappa$
<b>QUANTITY</b>	200 $\mu$ L, 1 mg/mL
<b>SOURCE</b>	Purified IgG from hybridoma supernatant
<b>FORMULATION</b>	PBS containing 50% Glycerol (pH 7.2). No preservative is contained.
<b>STORAGE</b>	This antibody solution is stable for one year from the date of purchase when stored at -20°C.

## APPLICATIONS

<u>RNP immunoprecipitation (RIP)</u>	15 $\mu$ g/500 $\mu$ L of cell extract from $1.0 \times 10^7$ cells/sample
<u>Western blotting</u>	0.5-1 $\mu$ g/mL
<u>Immunoprecipitation</u>	1 $\mu$ g/200 $\mu$ L of cell extract from $2.0 \times 10^6$ cells/sample

## SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cell	293T, HeLa, HL-60, Jurkat, Raji, A431	NIH/3T3, WR19L	NRK, PC12	CHO
Reactivity	+	+	+	+

**Entrez Gene ID** 6175 (P0, Human), 6176 (P1, Human), 6181 (P2, Human)  
11837 (P0, Mouse), 56040 (P1, Mouse), 67186 (P2, Mouse)

**REFERENCES**

- 1) Uchiumi, T., *et al.*, *J. Biol. Chem.* **265**, 89-95 (1990)
- 2) Towbin, H., *et al.*, *J. Biol. Chem.* **257**, 12709-12715 (1982)
- 3) Sun, K. H., *et al.*, *Reumatology* **40**, 750-756 (2001)

## RELATED PRODUCTS

For more information, please visit our web site at <https://ruo.mbl.co.jp/>.

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

### **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.

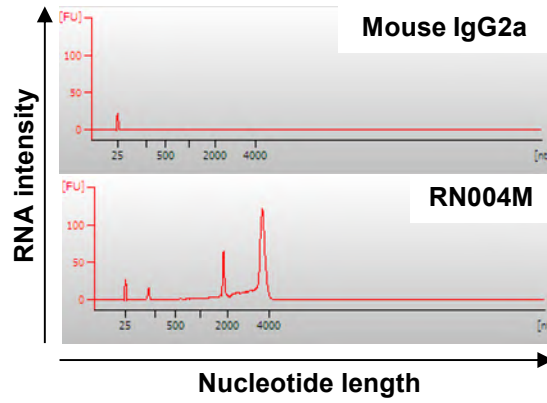
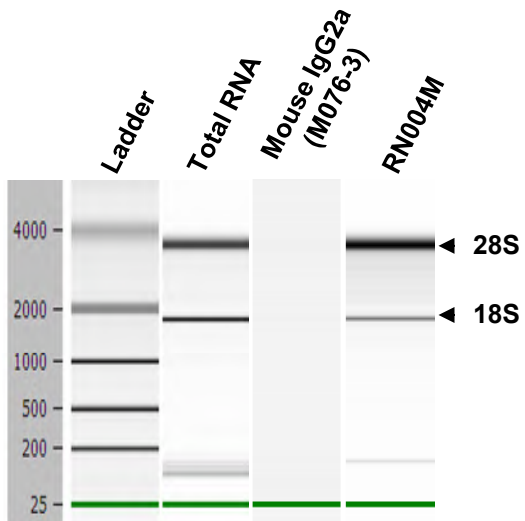
#### [Material Preparation]

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.
3. Antibody conjugated Protein A beads
  - A) Mix 25  $\mu$ L of 50% protein A agarose beads slurry resuspended in nuclease-free PBS with 1 mL of Wash Buffer (+), and then add Mouse IgG2a (code. M076-3) or Anti-Ribosomal P0/P1/P2 mAb at the concentration suggested in the **APPLICATIONS**. Incubate with gentle agitation for 1 hr. at 4°C.
  - B) During pre-clear steps (Protocol 3)), wash the beads 1 time with ice-cold Lysis Buffer (+) (centrifuge the tube at 2,000 x g for 1 min.). Carefully discard the supernatant using a pipettor without disturbing the beads and incubate at 4°C until just before use.

#### [Protocol]

- 1) Wash the cells ( $1.0 \times 10^7$  cells/sample) 4 times with PBS and resuspend them with 500  $\mu$ L of ice-cold Lysis Buffer (+) containing appropriate protease inhibitors, RNase inhibitors, and DTT. Vortex thoroughly, then incubate on ice for 10 min.
- 2) Centrifuge the tube at 12,000 x g for 5 min. 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 hr.
- 4) Centrifuge the tube at 2,000 x g for 1 min. at 4°C and transfer the supernatant to the tube containing antibody conjugated beads, then incubate with gentle agitation for 3 hr. at 4°C.
- 5) Wash the beads 4 times with Wash Buffer (+) (centrifuge the tube at 2,000 x g for 1 min.).
- 6) Add 400  $\mu$ L of Master mix solution (Solution I: Solution II = 10  $\mu$ L: 390  $\mu$ L). Vortex thoroughly, then spin-down.
- 7) Add 250  $\mu$ L of Solution III. Vortex thoroughly. Centrifuge the tube at 2,000 x g for 2 min.
- 8) Transfer the supernatant to the tube containing 2  $\mu$ L of Solution IV.
- 9) Add 600  $\mu$ L of ice-cold 2-propanol and place at -20°C for 20 min. Centrifuge the tube at 12,000 x g for 10 min.
- 10) Wash the pellet 2 times with 0.5 mL of ice-cold 70% ethanol and let the pellet dry for 5-15 min.
- 11) Dissolve the pellet in nuclease-free water.
- 12) Quantify the isolated RNA using NanoDrop (Thermo Fisher Scientific Inc.) and check the quality of RNA with Bioanalyzer (Agilent Technologies, Inc.).

(Positive control for RNP immunoprecipitation; 293T)



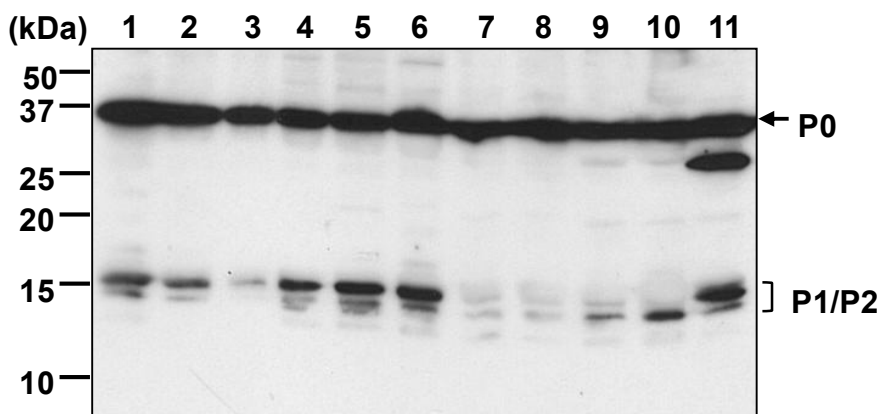
**Analysis of RNA with Bioanalyzer**

Average of the RNA Quantity (n=2)	
Antibody	RNA (ng)
Mouse IgG2a	56.2
RN004M	4891.4
Total RNA	163215.0

### **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, then sonicate briefly (up to 10 sec.).
- 2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
- 3) Boil the samples for 3 min. and centrifuge. Load 10  $\mu$ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (15% acrylamide) for electrophoresis.
- 4) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm<sup>2</sup> for 1 hr. 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.
- 5) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 7) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) [5 min. x 3 times].
- 8) Incubate the membrane with the 1:10,000 of anti-IgG (H+L chain) (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 9) Wash the membrane with PBS-T (5 min. x 3 times).
- 10) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 11) Expose to an X-ray film in a dark room for 30 sec. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; 293T, HeLa, HL-60, Jurkat, Raji, A431, NIH/3T3, WR19L, NRK, PC12 and CHO)



#### ***Western blot analysis of Ribosomal P0/P1/P2***

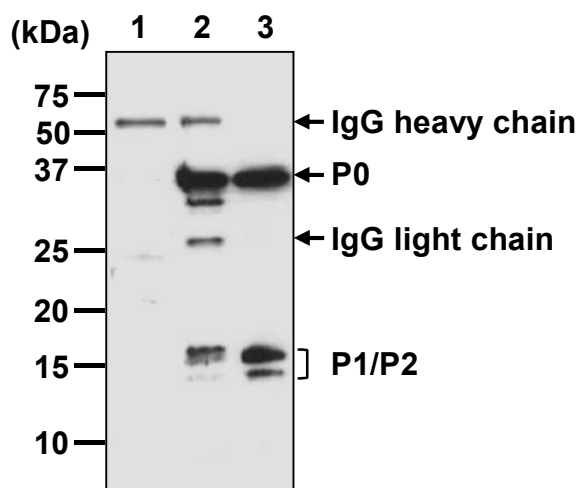
- Lane 1: 293T
- Lane 2: HeLa
- Lane 3: HL-60
- Lane 4: Jurkat
- Lane 5: Raji
- Lane 6: A431
- Lane 7: NIH/3T3
- Lane 8: WR19L
- Lane 9: NRK
- Lane 10: PC12
- Lane 11: CHO

Immunoblotted with RN004M

### **Immunoprecipitation**

- 1) Wash  $1 \times 10^7$  cells 2 times with PBS and resuspend them with 1 mL of Extraction buffer (50 mM Tris-HCl pH 7.4, 150mM NaCl, 0.05% NP-40) containing appropriate protease inhibitors, then sonicate briefly (up to 15 seconds).
- 2) Incubate the tube for 15 min. on ice.
- 3) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
- 4) Mix 20  $\mu$ L of 50% protein A agarose beads slurry resuspended in 400  $\mu$ L of IP buffer (10 mM Tris-HCl pH 8.0, 500mM NaCl, 0.1% NP-40) with primary antibody as suggested in the **APPLICATIONS**. Incubate with gentle agitation for 1 hr. at room temperature.
- 5) Wash the beads 3 times with IP Buffer.
- 6) Add 200  $\mu$ L of cell lysate (prepared sample from step 3)), then incubate with gentle agitation for 1 hr. at room temperature.
- 7) Wash the beads 6 times with 1 mL of Extraction buffer.
- 8) Resuspend the beads in 20  $\mu$ L of Laemmli's sample buffer, boil for 3 min. and centrifuge.
- 9) Load 10  $\mu$ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (15% acrylamide) for electrophoresis.
- 10) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm<sup>2</sup> for 1 hr. 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.
- 11) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 12) Incubate the membrane with 0.5  $\mu$ g/ml of anti-Ribosomal P0/P1/P2 mAb (MBL; code no. RN004M) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 13) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) [5 min. x 3 times].
- 14) Incubate the membrane with the 1:10,000 of anti-IgG (H+L chain) (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 15) Wash the membrane with PBS-T (5 min. x 3 times).
- 16) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 17) Expose to an X-ray film in a dark room for 1 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; 293T)



### ***Immunoprecipitation of Ribosomal P0/P1/P2 from 293T cells***

Lane 1: Mouse IgG2a (M076-3)  
Lane 2: RN004M  
Lane 3: Input (whole cell lysate)

Immunoblotted with RN004M