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RiboCluster Profiler™

RIP-Certified Antibody

Anti-Ribosomal P0/P1/P2 mAb

CODE No. RN004M

CLONALITY Monoclonal
CLONE 9D5
ISOTYPE Mouse IgG2a κ
QUANTITY 200 µL, 1 mg/mL

SOURCE Purified IgG from hybridoma supernatant
FORMULATION 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.

APPLICATIONS

RNP immunoprecipitation (RIP) 15 µg/500 µL of cell extract from 1.0 x 10^7 cells/sample
Western blotting 0.5-1 µg/mL
Immunoprecipitation 1 µg/200 µL of cell extract from 2.0 x 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

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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.

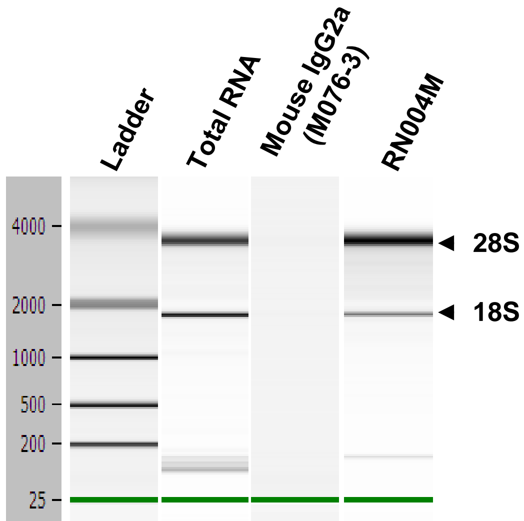
[Material Preparation]

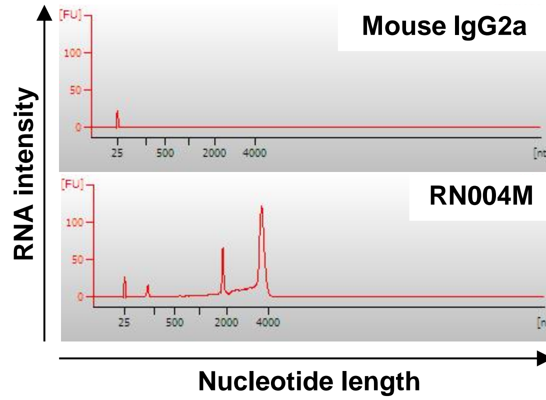
1. Lysis Buffer (+)
   Before using the Lysis Buffer, protease inhibitors, RNase inhibitors, and DTT are added to the Lysis Buffer at the appropriate concentration.
2. Wash Buffer (+)
   Before using the Wash Buffer, DTT is added to the Wash Buffer at the appropriate concentration.
3. Antibody conjugated Protein A beads
   A) Mix 25 μL 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 x 10^7 cells/sample) 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 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 μL 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 μL of Master mix solution (Solution I: Solution II = 10 μL: 390 μL). Vortex thoroughly, then spin-down.
7) Add 250 μL 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 μL of Solution IV.
9) Add 600 μL 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)





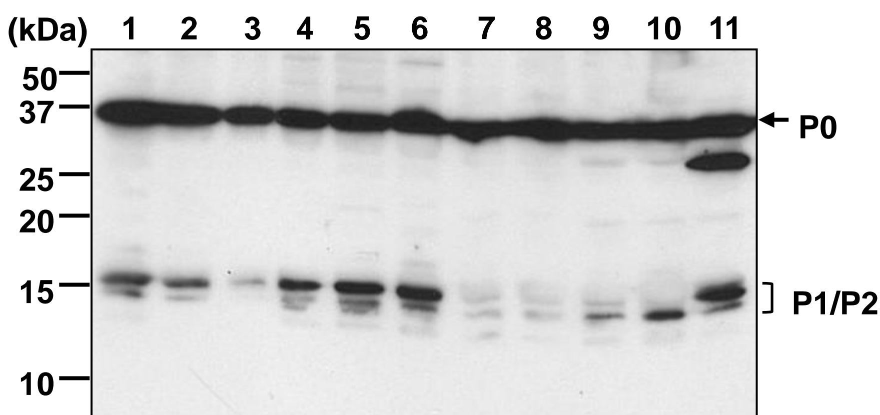
Analysis of RNA with Bioanalyzer

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 x 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 µL 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^2 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

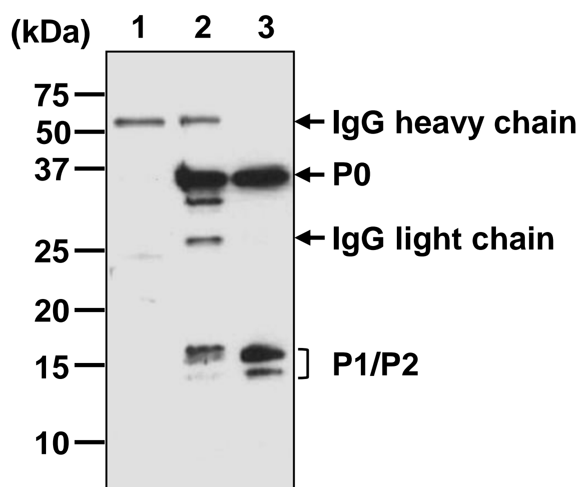
Lane 1: 293T
Lane 2: HeLa
Lane 3: HL-60
Lane 4: Jurkat
Lane 5: Raji
Lane 6: A431
Lane 7: NIH/3T3
Lane 8: WR19L
Lane 9: NRK
Lane 10: PC12
Lane 11: CHO

Immunoblotted with RN004M

Immunoprecipitation

1) Wash 1 x 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 µL of 50% protein A agarose beads slurry resuspended in 400 µL 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 µL 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 µL of Laemmli's sample buffer, boil for 3 min. and centrifuge.
9) Load 10 µL 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^2 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 µg/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