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

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
FORMURATION	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 for chemiluminescence detection system
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)

For more information, please visit our web site **http://ruo.mbl.co.jp/je/rip-assay/**

LICENSING OPPORTUNITY: The RIP-Assay uses patented technology (US patent No. 6,635,422, US patent No. 7,504,210, JP patent No. 5,002,105) 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

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL http://ruo.mbl.co.jp/je/rip-assay/
e-mail support@mbl.co.jp, TEL 052-238-1904

RELATED PRODUCTS

RIP-Assay Kit

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

RIP-Certified Antibody

RN004M	Anti-Ribosomal P0/P1/P2 mAb
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
RN001M	Anti-IGF2BP1 (IMP1) mAb (6H6)
RN003M	Anti-EIF2C2 (AGO2) mAb (1B1-E2H5)
RN005M	Anti-EIF2C2 (AGO2) mAb (2A8)

RBP Antibody

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

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 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
RN002MW	Anti-CUGBP1 mAb (3B1)

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

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.

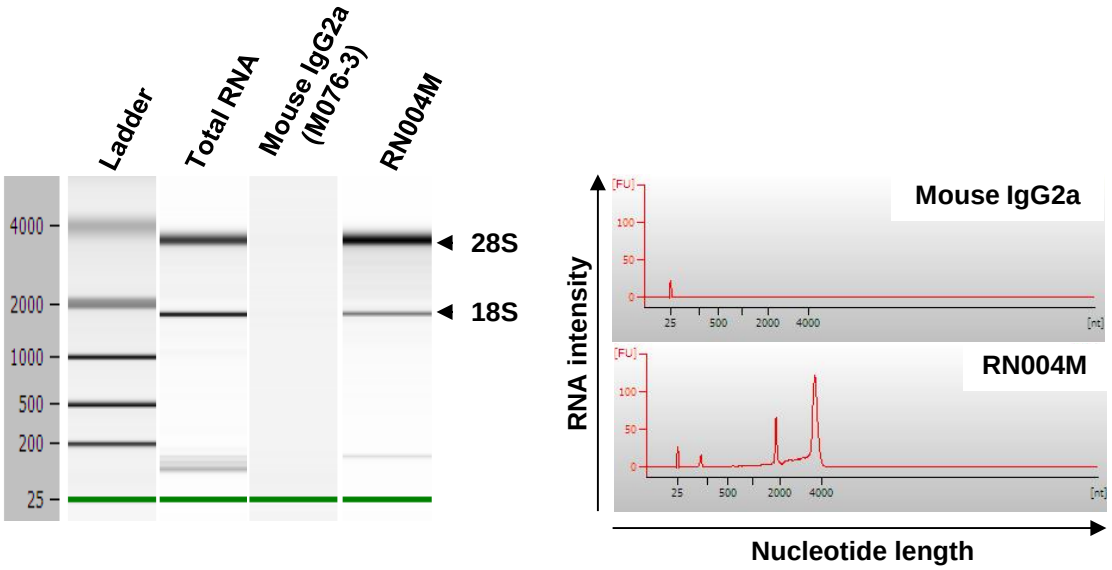
[Material Preparation]

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)



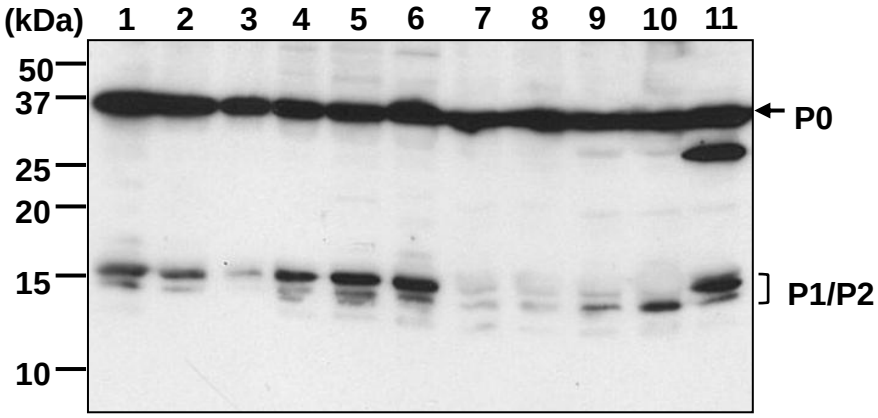
Analysis of RNA with Bioanalyzer

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

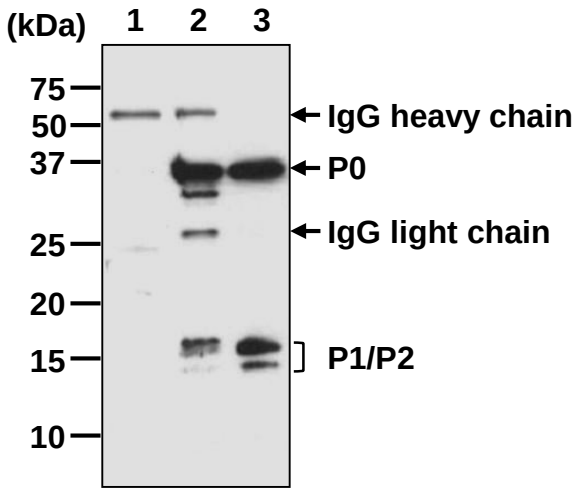
Lane 1: 293T
Lane 2: HeLa
Lane 3: HL-60
Lane 4: Jurkat
Lane 5: Raji
Lane 6: A431
Lane 7: NIH/3T3
Lane 8: WR19L
Lane 9: NRK
Lane 10: PC12
Lane 11: CHO

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