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

RiboCluster Profiler™ RBP Antibody

Anti-Nono (P54NRB) mAb

CODE No. RN013MW

CLONALITY Monoclonal
CLONE C5
ISOTYPE Mouse IgG2a κ
QUANTITY 100 µL, 1 mg/mL

SOURCE Purified IgG from hybridoma supernatant
IMMUNOGEN KLH-conjugated synthetic peptide, CPPAFNRPAPGAE (corresponding to amino acid residues 453-464 of mouse Nono)
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-CONFIRMED

Western blotting 1 µg/mL for chemiluminescence detection system
Immunoprecipitation 2-5 µg/sample
Immunohistochemistry 5 µg/mL
Heat treatment for paraffin embedded section: Microwave oven; 100°C for 20 min. in 10 mM citrate buffer (pH 6.2)
Immunocytochemistry 10 µg/mL

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cells	HeLa, Jurkat	NIH/3T3, MEF, WR19L, C2C12	Rat1	CHO
Reactivity	-	+	+	+

Entrez Gene ID 53610 (Mouse), 317259 (Rat), 100757453 (Hamster)

REFERENCES

1) Imamura, K., *et al., Mol. Cell* **53**, 393-406 (2014)
2) Hirose, T., *et al., Mol. Biol. Cell.* **25**, 169-183 (2014)
3) Elzbieta, K., *et al., Mol. Cell. Biol.* **32**, 4585-4594 (2012)
4) Nakagawa, S. and Hirose, T., *Cell Mol. Life Sci.* **69**, 3027–3036 (2012)
5) Nakagawa, S., *et al., J. Cell Biol.* **193**, 31-39 (2011)
6) Kuwahara, S., *et al., Biol. Reprod.* **75**, 352-359 (2006)
7) Fox, A. H., *et al., Curr. Biol.* **12**, 13-25 (2002)

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

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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

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)
RN004M	Anti-Ribosomal P0/P1/P2 mAb (9D5)
RN005M	Anti-EIF2C2 (AGO2) mAb (2A8)
RN006M	Anti-EIF4E mAb (C107-3-5)
RN007M	Anti-ELAVL1 (HuR) mAb (C67-1)
RN009M	Anti-PABPC1 mAb (10E10)

RBP Antibody

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

RN012MW	Anti-Coil (Coilin) mAb (#4)
RN013MW	Anti-Nono (P54NRB) mAb (C5)
RN014MW	Anti-SFPQ (PSF) mAb (C23)
RN015MW	Anti-PSPC1 (PSP1) mAb (1L4)
RN023PW	Anti-PABPN1 pAb
RN042PW	Anti-MBNL1 pAb
RN043PW	Anti-NOVA1 (Human) pAb
RN044PW	Anti-NOVA2 (Human) pAb
RN047PW	Anti-PTBP2 pAb
RN052PW	Anti-HNRNPC pAb
RN057PW	Anti-TARBP1 pAb
RN058PW	Anti-TARBP2 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
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
RN100PW	Anti-EXOSC5 (RRP46) (Human) pAb
RN101PW	Anti-FBL (Fibrillarin) pAb
RN102PW	Anti-GEMIN2 (Human) pAb
RN103PW	Anti-NCBP1 (CBP80) pAb
RN106PW	Anti-SFPQ (PSF) pAb
RN107PW	Anti-TARDBP (TDP-43) pAb
RN109PW	Anti-XRN1 (Human) pAb
RN113PW	Anti-DHX36 (RHAU) pAb
RN114PW	Anti-HNRNPA1 pAb
RN116PW	Anti-DDX39B (UAP56) pAb
RN117PW	Anti-CCAR2 (DBC1) pAb
RN121PW	Anti-FTO (Human) pAb

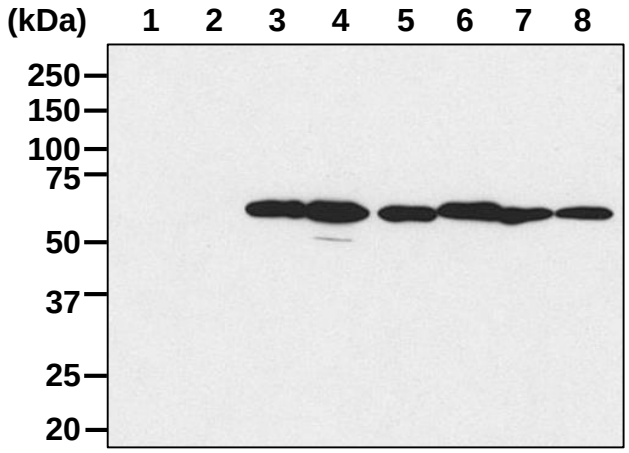
M019-3	Anti-Nucleolin mAb (4E2)
K0196-3	Anti-PML (Mouse) mAb (36-1-104)
M041-3	Anti-PML (Human) mAb (1B9)
PM001	Anti-PML (Human) pAb
PM064	Anti-Lamin B1 pAb

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

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 (12.5% 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) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) [5 min. x 3 times].
7) 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.)
8) Wash the membrane with PBS-T (5 min. x 3 times).
9) Incubate the membrane with the 1:10,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
10) Wash the membrane with PBS-T (5 min. x 3 times).
11) 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.
12) Expose to an X-ray film in a dark room for 3 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; NIH/3T3, MEF, WR19L, Rat1, C2C12 and CHO)



Western blot analysis of Nono (P54NRB)

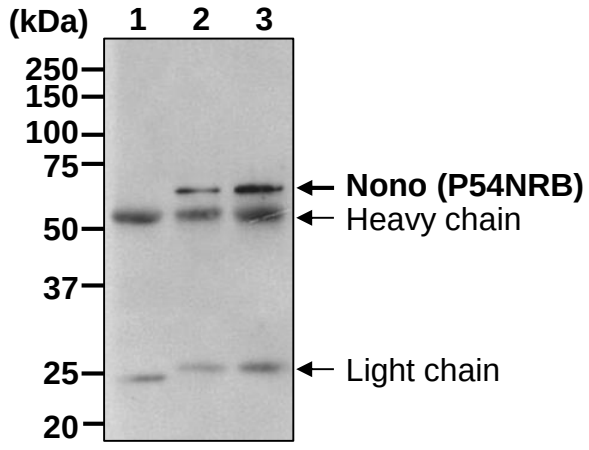
Lane 1: HeLa
Lane 2: Jurkat
Lane 3: NIH/3T3
Lane 4: MEF
Lane 5: WR19L
Lane 6: Rat1
Lane 7: C2C12
Lane 8: CHO

Immunoblotted with Anti-Nono (P54NRB) mAb (RN013MW)

Immunoprecipitation

1) Wash 1 x 10^7 cells 3 times with PBS and suspend them with 1 mL of Extraction buffer [50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.05% NP-40] containing appropriate protease inhibitors, then sonicate briefly (up to 15 sec.).
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), 500 mM 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 1 mL of IP buffer.
6) Add 250 µL of cell lysate (prepared sample from step 3), then incubate with gentle agitation overnight at 4°C.
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 (12.5% 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% methanol). 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) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
13) Incubate the membrane with 1 µg/mL of Anti-Nono (P54NRB) mAb (MBL; code no. RN013MW) 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.)
14) Wash the membrane with PBS-T (5 min. x 3 times).
15) Incubate the membrane with 1:1,000 of Mouse TrueBlot® ULTRA: Anti-Mouse Ig HRP (Rockland Immunochemicals; code no. 18-8817-31) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
16) Wash the membrane with PBS-T (5 min. x 3 times).
17) 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 a paper towel, and seal it in a plastic wrap.
    Expose to an X-ray film in a dark room for 5 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; NIH/3T3)



Immunoprecipitation of Nono (P54NRB) from NIH/3T3 cells

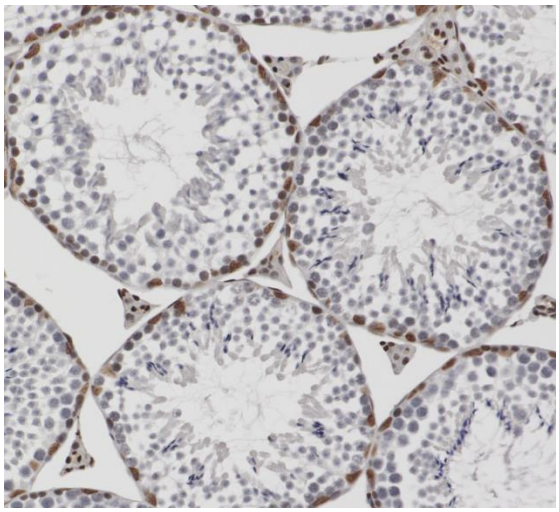
Lane 1: IP with 5 µg of Mouse IgG2a (isotype control) (M076-3)
Lane 2: IP with 2 µg of Anti-Nono (P54NRB) mAb (RN013MW)
Lane 3: IP with 5 µg of Anti-Nono (P54NRB) mAb (RN013MW)

Immunoblotted with RN013MW

Immunohistochemical staining for formalin fixed paraffin-embedded section

1) Deparaffinize the sections with Xylene 3 times for 5 min. each.
2) Wash the slides with Ethanol 3 times for 5 min. each.
3) Wash the slides with PBS 3 times for 5 min. each.
4) Remove the slides from PBS and heat-treated with 10 mM Citrate buffer (pH 6.2) for 20 min. at 100°C using microwave oven.
5) Let the slides cool down at room temperature in the Citrate buffer.
6) Remove the slides from the Citrate buffer and block endogenous peroxidase with 3% H_2O_2 in PBS for 10 min.
7) Wash the slides with PBS 2 times for 5 min. each.
8) 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 min. at room temperature (20~25°C) to block non-specific staining. Do not wash.
9) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with blocking buffer as suggested in the **APPLICATIONS** for 1 hr. at 4°C. (The concentration of antibody will depend on the conditions.)
10) Wash the slides 3 times in PBS for 5 min. each.
11) Wipe gently around each section and cover tissues with Histostar (Ms + Rb) (MBL; code no. 8460). Incubate for 1 hr. at room temperature.
12) Wash the slides 3 times in PBS for 5 min. each.
13) Visualize by reacting for 10 min. with Histostar DAB (MBL; code no. 8469) at room temperature. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
14) Wash the slides in water for 5 min.
15) Counter stain in hematoxylin for 1 min., wash the slides 3 times in water for 5 min. each, and then immerse the slides in PBS for 5 min.
16) Dehydrate by immersing in Ethanol 3 times for 3 min. each, followed by immersing in Xylene 3 times for 3 min. each. Now ready for mounting.

(Positive control for Immunohistochemistry; Mouse testis)



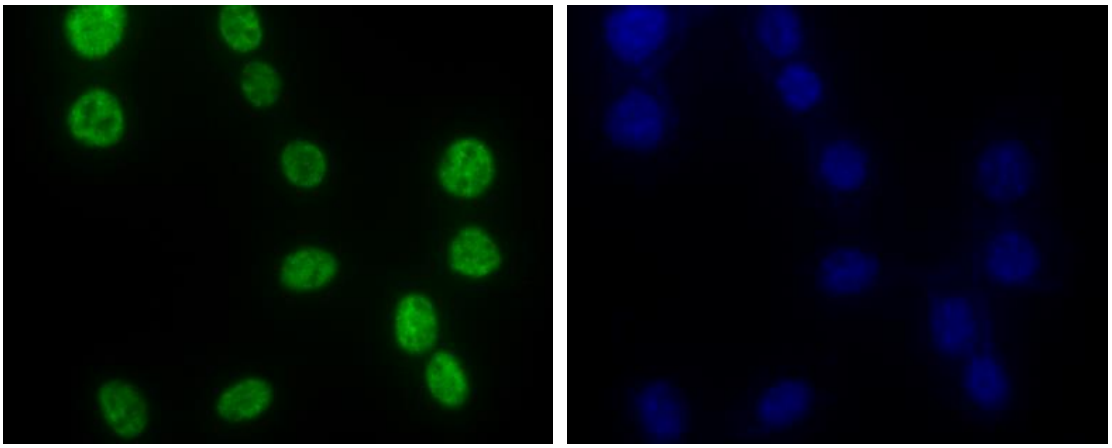
Immunohistochemical detection of Nono (P54NRB) in mouse testis

Brown: Anti-Nono (P54NRB) mAb (RN013MW)
Blue: Hematoxylin

Immunocytochemistry

1) Spread the cells on a glass slide, then incubate in a CO_2 incubator for one night.
2) Remove the culture supernatant by careful aspiration.
3) Wash the slide 2 times with PBS.
4) Fix the cells with 4% paraformaldehyde (PFA)/PBS for 10 min. at room temperature (20~25°C).
5) Wash the slide 3 times with PBS.
6) Permeabilize the cells with 0.2% Triton X-100/PBS for 10 min. at room temperature.
7) Wash the slide 3 times with PBS.
8) Tip off PBS and add the primary antibody diluted with 2% fetal calf serum (FCS)/PBS as suggested in the **APPLICATIONS** onto the cells. Incubate for 30 min. at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
9) Wash the slide 3 times with PBS.
10) Add 200 µL of 1:500 Alexa Fluor® 488 Goat Anti-mouse IgG (Invitrogen; code no. A11001) diluted with PBS onto the cells. Incubate for 30 min. at room temperature. Keep out light by aluminum foil.
11) Wash the slide 3 times with PBS.
12) Wipe excess liquid from the slide but take care not to touch the cells. Never leave the cells to dry.
13) Counterstain with DAPI for 5 min. at room temperature.
14) Wash the slide 1 time with PBS.
15) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; NIH/3T3)



Immunocytochemical detection of Nono (P54NRB) in NIH/3T3 cells
Green: Anti-Nono (P54NRB) mAb (RN013MW)
Blue: DAPI