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

Anti-NONO (P54NRB) pAb

CODE No. RN092PW

CLONALITY Polyclonal
ISOTYPE Rabbit Ig, affinity purified
QUANTITY 100 µL, 1 mg/mL

SOURCE Purified Ig from rabbit serum
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	1:1,000 for chemiluminescence detection system
Immunoprecipitation	5 µL/500 µL of cell extract from 2 x 10^7 cells

APPLICATION-UNDER EVALUATION

Immunocytochemistry	1:100

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cell	HeLa, A431, Jurkat, 293T	NIH/3T3	Rat1	CHO
Reactivity	+	+	+	+

Entrez Gene ID 4841 (Human), 53610 (Mouse), 317259 (Rat)

For more information, please visit our web site **https://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) 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 https://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

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/Musashi1 (polyclonal)
RN011P	Anti-PTBP1 (polyclonal)
RN012P	Anti-STAU1 (polyclonal)
RN013P	Anti-STAU2 (polyclonal)
RN014P	Anti-TIA1 (polyclonal)
RN015P	Anti-YBX1 (polyclonal)
RN016P	Anti-FMR1 (polyclonal)
RN017P	Anti-FXR1 (polyclonal)
RN018P	Anti-FXR2 (polyclonal)
RN019P	Anti-HNRNPK (polyclonal)
RN020P	Anti-ILF3 (polyclonal)
RN021P	Anti-KHDRBS1 (polyclonal)
RN022P	Anti-PABPC4 (polyclonal)
RN024P	Anti-PCBP1 (polyclonal)
RN025P	Anti-PCBP2 (polyclonal)
RN026P	Anti-PUM1 (polyclonal)
RN027P	Anti-PUM2 (polyclonal)
RN028P	Anti-EIF2C1/AGO1 (polyclonal)
RN032P	Anti-CIRBP (polyclonal)
RN033P	Anti-TNRC6A/GW182 (polyclonal)
RN037P	Anti-AUH (polyclonal)
RN038P	Anti-CPEB1 (polyclonal)
RN041P	Anti-KHDRBS2/SLM1 (polyclonal)
RN045P	Anti-SLBP (polyclonal)
RN001M	Anti-IGF2BP1/IMP1 (6H6)
RN003M	Anti-EIF2C2/AGO2 (1B1-E2H5)

RBP Antibody

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

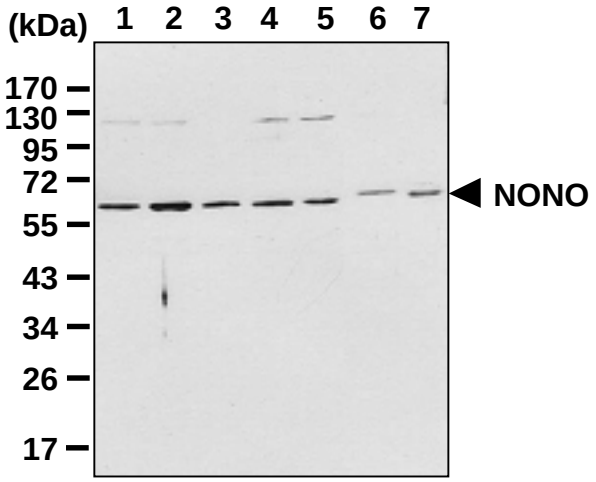
RN023PW	Anti-PABPN1 (polyclonal)
RN028PW	Anti-EIF2C1/AGO1 (polyclonal)
RN029PW	Anti-EIF2C2/AGO2 (polyclonal)
RN030PW	Anti-DICER1 (polyclonal)
RN031PW	Anti-ZFP36 (polyclonal)
RN034PW	Anti-CUGBP1 (polyclonal)
RN035PW	Anti-CUGBP2 (polyclonal)
RN036PW	Anti-ACO1/IRP1 (polyclonal)
RN039PW	Anti-CPEB2 (polyclonal)
RN040PW	Anti-CPEB4 (polyclonal)
RN042PW	Anti-MBNL1 (polyclonal)
RN043PW	Anti-NOVA1 (polyclonal)
RN044PW	Anti-NOVA2 (polyclonal)
RN046PW	Anti-SYNCRIP/HNRNPQ (polyclonal)
RN047PW	Anti-PTBP2 (polyclonal)
RN048PW	Anti-G3BP1 (polyclonal)
RN049PW	Anti-G3BP2 (polyclonal)
RN050PW	Anti-GRSF1 (polyclonal)
RN051PW	Anti-HDLBP/Vigilin (polyclonal)
RN052PW	Anti-HNRNPC (polyclonal)
RN053PW	Anti-PAIP1 (polyclonal)
RN054PW	Anti-PCBP3 (polyclonal)
RN055PW	Anti-AIMP1/SCYE1 (polyclonal)
RN056PW	Anti-SERBP1 (polyclonal)
RN057PW	Anti-TARBP1 (polyclonal)
RN058PW	Anti-TARBP2 (polyclonal)
RN059PW	Anti-TIAL1 (polyclonal)
RN060PW	Anti-HNRNPD/AUF1 (polyclonal)
RN061PW	Anti-HNRNPA0 (polyclonal)
RN062PW	Anti-DGCR8 (polyclonal)
RN063PW	Anti-DHX9 (polyclonal)
RN064PW	Anti-FUSIP1 (polyclonal)
RN065PW	Anti-KHSRP (polyclonal)
RN066PW	Anti-KIAA0020 (polyclonal)
RN067PW	Anti-PPP1R10 (polyclonal)
RN068PW	Anti-PPP1R8 (polyclonal)
RN069PW	Anti-RBM14 (polyclonal)
RN070PW	Anti-RPS10 (polyclonal)
RN071PW	Anti-RPS19 (polyclonal)
RN072PW	Anti-RPS6 (polyclonal)
RN073PW	Anti-RPS9 (polyclonal)
RN074PW	Anti-SSB (polyclonal)
RN075PW	Anti-PPARGC1B (polyclonal)
RN076PW	Anti-PPRC1 (polyclonal)
RN077PW	Anti-SMN1 (polyclonal)
RN078PW	Anti-SMNDC1 (polyclonal)
RN079PW	Anti-SRSF7/9G8 (polyclonal)
RN080PW	Anti-SRSF3/SRp20 (polyclonal)
RN081PW	Anti-SRSF9/SRp30c (polyclonal)
RN082PW	Anti-SRSF5/SRP40 (polyclonal)
RN083PW	Anti-AQR/IBP160 (polyclonal)
RN084PW	Anti-SRRM1/SRM160 (polyclonal)
RN085PW	Anti-U2AF1 (polyclonal)
RN086PW	Anti-U2AF2 (polyclonal)
RN087PW	Anti-THOC4 (polyclonal)
RN088PW	Anti-NXF1/TAP (polyclonal)
RN089PW	Anti-MAGOH (polyclonal)
RN090PW	Anti-DDX21 (polyclonal)
RN091PW	Anti-DDX23 (polyclonal)
RN092PW	Anti-NONO/p54nrb (polyclonal)
RN093PW	Anti-PRPF4 (polyclonal)
RN094PW	Anti-PRPF8 (polyclonal)
RN095PW	Anti-SNRNP200 (polyclonal)
RN096PW	Anti-SNRNP40 (polyclonal)
RN097PW	Anti-SNRNP70 (polyclonal)
RN002MW	Anti-CUGBP1 (3B1)
RN003MW	Anti-EIF2C2/AGO2 (1B1-E2H5)

For the latest information of RiboCluster Profiler™, please visit our website at https://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 20 sec.).
2) 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.
3) 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.
4) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for overnight at 4°C.
5) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
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 (10 min. x 3 times).
8) Incubate the membrane with the 1:5,000 anti-IgG (Rabbit)-HRP (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
9) Wash the membrane with PBS-T (10 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 3 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; HeLa, 293T, A431, Jurkat, NIH/3T3, Rat1 and CHO)



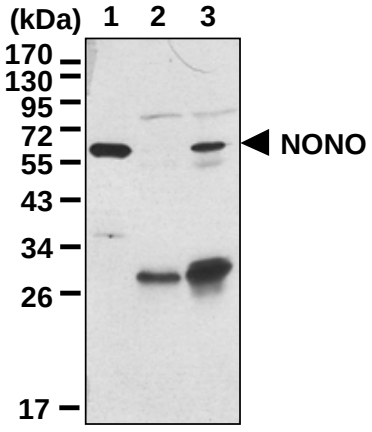
Western blot analysis of NONO

Lane 1: HeLa
Lane 2: 293T
Lane 3: A431
Lane 4: Jurkat
Lane 5: NIH/3T3
Lane 6: Rat1
Lane 7: CHO
Immunoblotted with RN092PW

Immunoprecipitation

1) Wash 4 x 10^7 cells 2 times with PBS and resuspend them with 1 mL of ice-cold Lysis buffer (150 mM NaCl, 20 mM Tris-HCl, pH 8.0, 0.1% NP-40, 10 mM EDTA) containing appropriate protease inhibitors and 1.5 mM DTT. Vortex thoroughly, then incubate it on ice for 10 min.
2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and discard the supernatant.
3) Wash the pellet 3 times with PBS and resuspend them with 500 µL RIPA buffer, then sonicate briefly.
4) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another fresh tube.
5) Add 500 µL of ice-cold Lysis buffer into the supernatant. Mix by pipetting up and down.
6) Add 40 µL of 50% protein G agarose beads slurry resuspended in Lysis Buffer into the sample (prepared from step 5). Incubate it at 4°C with rotating for 1 hr.
7) Centrifuge the tube at 2,000 x g for 2 minutes at 4°C and transfer the supernatant to another tube (precleared sample).
8) Mix 20 µL of 50% protein G agarose beads slurry resuspended in PBS with normal rabbit IgG (RIP-Assay Kit) or anti-NONO pAb at the amount of suggested in the **APPLICATIONS,** then add 1 mL of Lysis Buffer into each tube. Incubate with gentle agitation for 1 hr. at 4°C.
9) Wash the beads once with 500 µL of ice-cold Lysis Buffer (centrifuge the tube at 2,000 x g for 1 min.). Carefully discard the supernatant using a pipette or without disturbing the beads.
10) Add 500 µL of nuclear extract (the sample from step 7), then incubate with gentle agitation for 3 hr. at 4°C.
11) Wash the beads 4 times with Wash Buffer (centrifuge the tube at 2,000 x g for 1 min.).
12) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3 min., and centrifuge for 5 min. Use 20 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
13) 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.
14) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature, or overnight at 4°C.
15) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
16) 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.)
17) Wash the membrane with PBS-T (10 min. x 3 times).
18) Incubate the membrane with the 1:1,000 Rabbit TrueBlot® anti-Rabbit IgG-HRP (eBioscience; code no. 18-8816-33) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
19) Wash the membrane with PBS-T (10 min. x 3 times).
20) 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.
21) 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 control for Immunoprecipitation; 293T nuclear extract)

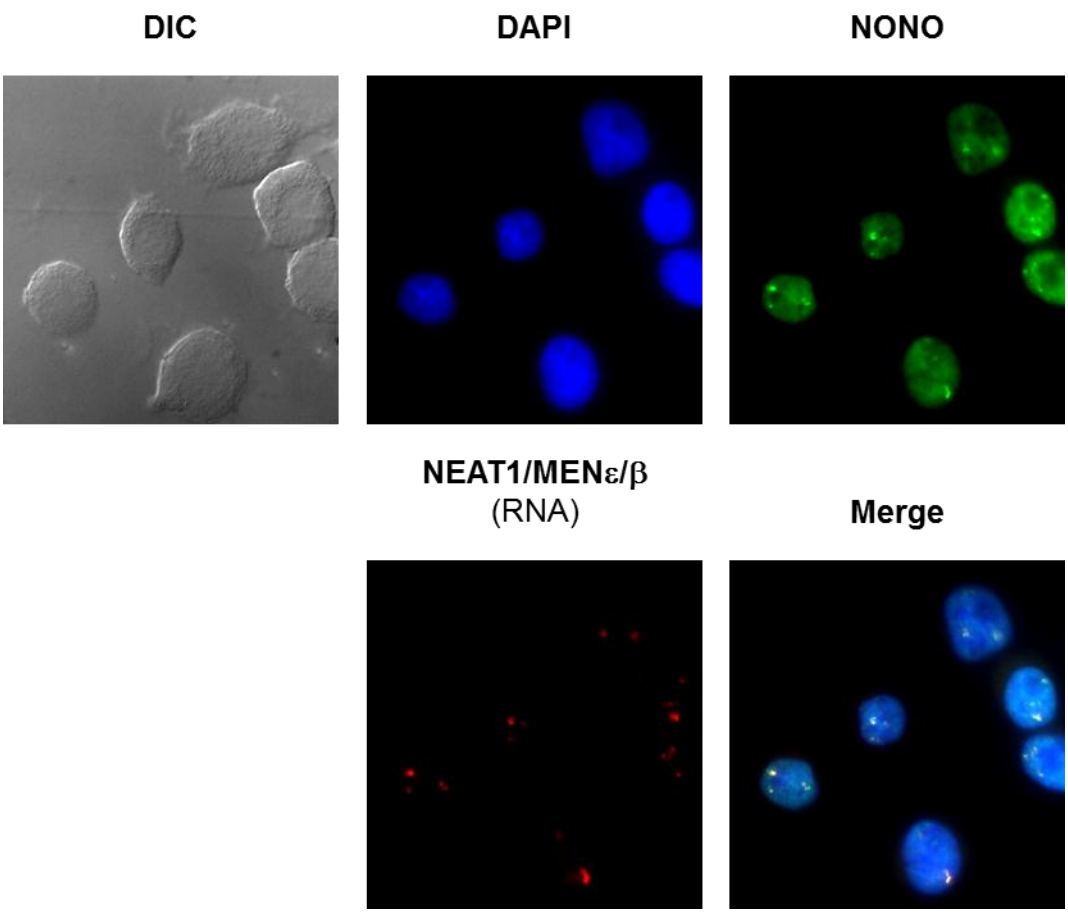


Immunoprecipitation of NONO from 293T

Lane 1: Input
Lane 1: IP with normal rabbit IgG
Lane 2: IP with RN092PW

Immunoblotted with RN092PW

Immunocytochemistry (Under evaluation)



Immunocytochemical detection of NONO from HeLa