

RiboCluster Profiler™

RBP Antibody

Anti-HNRNPA1 pAb

CODE No.	RN114PW
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

<u>Western blotting</u>	1:1,000 for chemiluminescence detection system
<u>Immunoprecipitation</u>	5 µL/500 µL of nuclear or whole cell extract from 1 x 10 ⁷ cells/sample

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cells	HeLa, HEK293T, Jurkat*, K562	NIH/3T3	Rat1	CHO
Reactivity	+	+	+	+ (weak)

*very weak reactivity

Entrez Gene ID 3178 (Human), 15382 (Mouse), 29578 (Rat), 100769923 (Hamster)

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@mbi.co.jp

RELATED PRODUCTS

RIP-Assay Kit

RN1001	RIP-Assay Kit
RN1005	RIP-Assay Kit for <i>microRNA</i>

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
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

RN008MW	Anti-ELAVL1 (HuR) mAb (C54-6)
RN010MW	Anti-PIWIL1 (MIWI) mAb (2D9)
RN023PW	Anti-PABPN1 pAb
RN030PW	Anti-DICER1 pAb
RN031PW	Anti-ZFP36 (TTP) pAb
RN046PW	Anti-SYNERIP (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
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 (SRSF10) pAb
RN065PW	Anti-KHSRP pAb
RN069PW	Anti-RBM14 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
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
RN098PW	Anti-EDC4 pAb
RN099PW	Anti-EIF4A1 pAb
RN100PW	Anti-EXOSC5 (RRP46) (Human) pAb
RN101PW	Anti-FBL (Fibrillar) pAb
RN102PW	Anti-GEMIN2 (Human) pAb
RN103PW	Anti-NCBP1 (CBP80) pAb
RN104PW	Anti-PAN2 (USP52) (Human) pAb
RN105PW	Anti-PARN pAb
RN106PW	Anti-SFPQ (PSF) pAb
RN107PW	Anti-TARDBP (TDP-43) pAb
RN108PW	Anti-UPF1 pAb
RN109PW	Anti-XRN1 (Human) pAb
RN110PW	Anti-CNOT7 (CAF1) pAb
RN111PW	Anti-ETF1 (eRF1) pAb
RN112PW	Anti-DCP1B (Human) pAb
RN113PW	Anti-DHX36 (RHAU) pAb
RN114PW	Anti-HNRNPA1 pAb
RN115PW	Anti-LIN28B (Human) pAb
RN116PW	Anti-DDX39B (UAP56) pAb
RN117PW	Anti-CCAR2 (DBC1) pAb

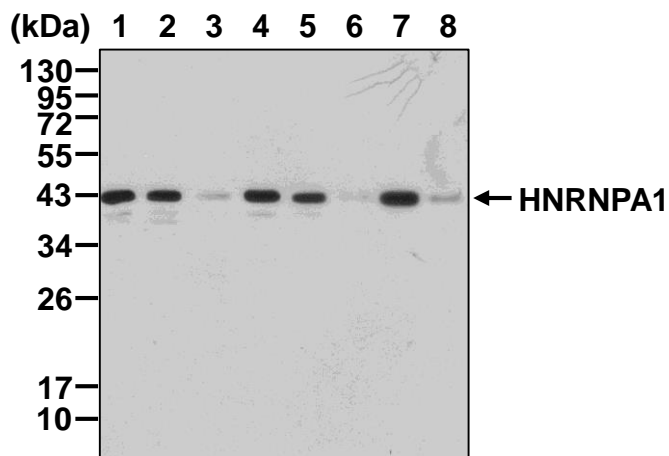
PD009	Anti-rck (p54) pAb
D216-3	Anti-hnRNP A1/B2 mAb (C20308)
PM064	Anti-Lamin B1 pAb
PM001	Anti-PML (Human) pAb
M041-3	Anti-PML (Human) mAb (1B9)
K0196-3	Anti-PML (Mouse) mAb (36-1-104)

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 1×10^7 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 μ L 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/cm² 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) 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 of Anti-IgG (Rabbit) pAb-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 settings. The condition for exposure and development may vary.

(Positive controls for Western blotting; HeLa, HEK293T, Jurkat, K562, NIH/3T3, Rat1 and CHO)



Western blot analysis of HNRNPA1

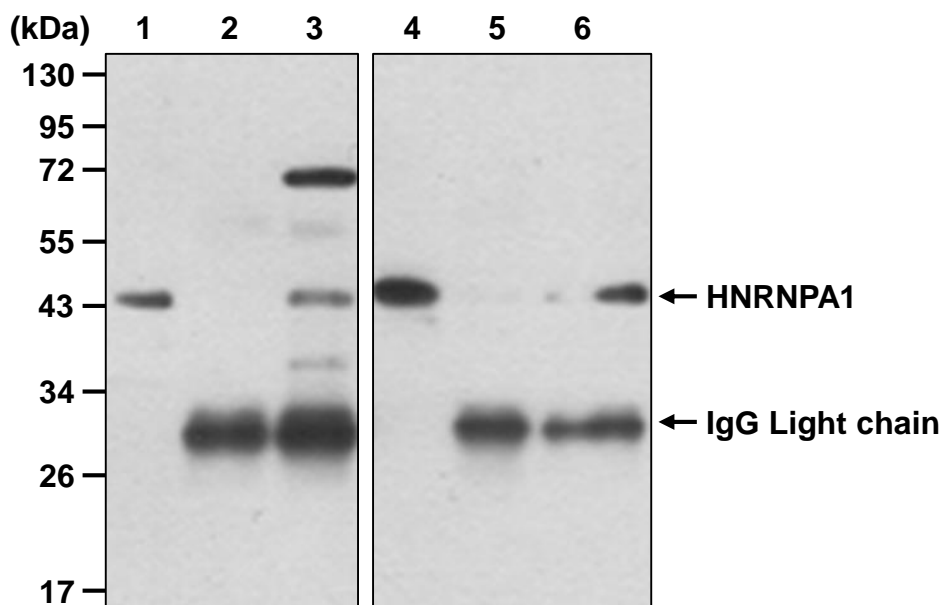
- Lane 1: HeLa
- Lane 2: HEK293T
- Lane 3: Jurkat
- Lane 4: K562
- Lane 5: NIH/3T3
- Lane 6: WR19L
- Lane 7: Rat1
- Lane 8: CHO

Immunoblotted with Anti-HNRNPA1 pAb (RN114PW)

Immunoprecipitation

- 1) Wash 2×10^7 cells 4 times with PBS and resuspend them with 1 mL of ice-cold Lysis Buffer (+) (MBL; code no. RN1001) containing appropriate protease inhibitors and DTT. Vortex thoroughly, then incubate on ice for 5-10 min.
- 2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube (total cell extract). If you don't need nuclear extract, please skip to step 6)
- 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 tube.
- 5) Add 500 µL of ice-cold Lysis Buffer into the supernatant. Mix well by pipetting up and down (nuclear extract).
- 6) Add 20 µL of 50% protein A agarose beads slurry resuspended in ice-cold Lysis Buffer (+) into the total cell extract and/or nuclear extract. Incubate it at 4°C with rotating for 1 hr.
- 7) Centrifuge the tube at 2,000 x g for 2 min. at 4°C and transfer the supernatant to another tube (precleared sample).
- 8) Mix 20 µL of 50% protein A agarose beads slurry resuspended in 1 mL of ice-cold Wash Buffer (+) (MBL; code no. RN1001) containing DTT at the appropriate concentration with Normal Rabbit IgG (RIP-Assay Kit) or Anti-HNRNPA1 pAb (MBL; code no. RN114PW) as suggested in the **APPLICATIONS**. Incubate at 4°C with rotating for 1 hr.
- 9) Wash the beads 1 time with ice-cold Lysis Buffer (+). Carefully discard the supernatant.
- 10) Add 500 µL of the precleared sample (prepared in step 7)) to the tube containing antibody conjugated beads, then incubate with gentle agitation for 2 hr. at 4°C.
- 11) Wash the beads 4 times with 500 µL of ice-cold Wash Buffer (+).
- 12) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3 min. and centrifuge. Use 10 µL/lane for the SDS-PAGE analysis. (See **SDS-PAGE & Western blotting**.) For 2nd antibody reaction, use 1:1,000 of True blot[®]: Anti-Rabbit IgG HRP (Rockland Immunochemicals; code no. 18-8816-31).

(Positive control for Immunoprecipitation; HEK293T)



Immunoprecipitation of HNRNPA1 from HEK293T cells

Lane 1-3: Total cell extract
Lane 4-6: Nuclear extract

Lane 1, 4: Input
Lane 2, 5: Normal Rabbit IgG
Lane 3, 6: Anti-HNRNPA1 pAb (RN114PW)

Immunoblotted with RN114PW