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

RiboCluster Profiler™ RBP Antibody

Anti-HNRNPD (AUF1) pAb

CODE No. RN060PW

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

Western blotting	1:1,000 for chemiluminescence detection system
Immunoprecipitation	5 µL/500 µL of cell extract from 2 x 10^7 cells/sample

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cells	293T, HeLa, Jurkat, K562	NIH/3T3, WR19L	Rat1	CHO
Reactivity	+	+	+	+

Entrez Gene ID 3184 (Human), Mouse (11991), Rat (79256), Hamster (100767887)

REFERENCE

1) Ishii, T., *et al.*, *Free Radic. Biol. Med.* **79C**, 109-116 (2014)

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 and 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/
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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 (Human) pAb
RN003P	Anti-EIF4G2 pAb
RN004P	Anti-ELAVL1 (HuR) pAb
RN005P	Anti-ELAVL2 (HuB) (Human) 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 (Human) pAb
RN012P	Anti-STAU1 (Human) pAb
RN013P	Anti-STAU2 (Human) pAb
RN015P	Anti-YBX1 pAb
RN019P	Anti-HNRNPK pAb
RN020P	Anti-ILF3 (Human) 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) (Human) 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) (Human) 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

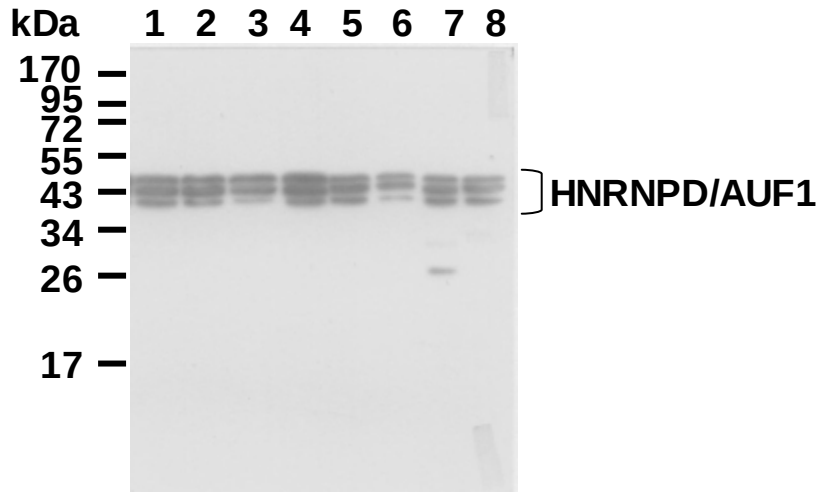
RN008MW	Anti-ELAVL1 (HuR) mAb (C54-6)
RN010MW	Anti-PIWIL1 (MIWI) mAb (2D9)
RN023PW	Anti-PABPN1 pAb
RN047PW	Anti-PTBP2 pAb
RN050PW	Anti-GRSF1 pAb
RN051PW	Anti-HDLBP (Vigilin) pAb
RN052PW	Anti-HNRNPC pAb
RN054PW	Anti-PCBP3 pAb
RN060PW	Anti-HNRNPD (AUF1) pAb
RN061PW	Anti-HNRNPA0 pAb
RN063PW	Anti-DHX9 pAb
RN064PW	Anti-FUSIP1 (SRSF10) pAb
RN065PW	Anti-KHSRP pAb
RN067PW	Anti-PPP1R10 pAb
RN068PW	Anti-PPP1R8 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 (Fibrillarin) 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
RN118PW	Anti-UPF3B pAb
RN119PW	Anti-GSPT2 (eRF3b) (Human) pAb
RN120PW	Anti-RBM8A (Y14) pAb
RN121PW	Anti-FTO (Human) pAb
RN122PW	Anti-ALKBH5 pAb
RN123PW	Anti-YTHDF2 pAb
RN124PW	Anti-RNMT (Human) pAb
RN125PW	Anti-HENMT1 pAb
RN126PW	Anti-AHCY (SAHH) pAb
RN127PW	Anti-NSUN2 (Human) pAb
RN128PW	Anti-TRMT6 (Human) pAb
RN129PW	Anti-DDX6 (RCK/p54) pAb
RN130PW	Anti-TRMT61A (Human) 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 20 sec.).
2) Boil the samples for 2 min. and centrifuge. Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel 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 manufacture's manual for precise transfer procedure.
4) 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.
5) Incubate the membrane with primary antibody diluted with 1% skimmed milk PBS (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.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (10 min. x 3 times).
7) Incubate the membrane with 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.
8) Wash the membrane with PBS-T (10 min. x 3 times).
9) 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.
10) 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; 293T, HeLa, Jurkat, K562, NIH/3T3, WR19L, Rat1 and CHO)



Western blot analysis of HNRNPD (AUF1)

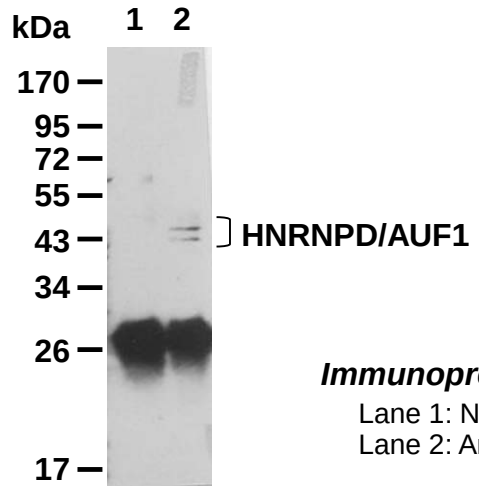
Lane 1: 293T
Lane 2: HeLa
Lane 3: Jurkat
Lane 4: K562
Lane 5: NIH/3T3
Lane 6: WR19L
Lane 7: Rat1
Lane 8: CHO

Immunoblotted with Anti-HNRNPD (AUF1) pAb (RN060PW)

Immunoprecipitation

1) Wash 4 x 10^7 cells 2 times with PBS and resuspend them with 1 mL of ice-cold Extraction Buffer [150 mM NaCl, 20 mM Tris-HCl, (pH 8.0), 0.1% NP-40, 10 mM EDTA] containing appropriate protease inhibitors. Vortex for 10 sec., then leave 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 40 µL of 50% protein A agarose beads slurry resuspended in Extraction 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 another tube (precleared sample).
5) Mix both 20 µL of 50% protein A agarose beads slurry resuspended in PBS and Normal Rabbit IgG (MBL; code no. PM035) or Anti-HNRNPD (AUF1) pAb (MBL; code no. RN060PW) at the amount of suggested in the **APPLICATIONS,** and then add 1 mL of Extraction Buffer into each tube. Incubate with gentle agitation for 1 hr. at 4°C.
6) Wash the beads once with ice-cold Extraction Buffer (centrifuge the tube at 2,000 x g for 1 min.). Carefully discard the supernatant using a pipettor without disturbing the beads.
7) Add 500 µL of cell lysate (precleared sample of step 4), then incubate with gentle agitation for 3 hr. at 4°C.
8) Wash the beads 4 times with Wash Buffer (centrifuge the tube at 2,000 x g for 1 min.).
9) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 min., and centrifuge for 5 min.
10) Load 20 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel for electrophoresis.
11) 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 manufacture's manual for precise transfer procedure.
12) 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.
13) 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.)
14) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (10 min. x 3 times).
15) Incubate the membrane with the 1:1,000 Rabbit True Blot HRP conjugated anti-Rabbit IgG (eBioscience; code no. 18-8816-33) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
16) Wash the membrane with PBS-T (10 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 paper towel, and seal it in plastic wrap.
18) 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)



Immunoprecipitation of HNRNPD (AUF1) from 293T cells
Lane 1: Normal Rabbit IgG (PM035)
Lane 2: Anti-HNRNPD (AUF1) pAb (RN060PW)