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

Anti-HNRNPD/AUF1

Code No.: RN060PW

SOURCE: rabbit polyclonal antibody, affinity purified
QUANTITY: 100 µL
FORMULATION: 1 mg/ml in 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

For application specific protocols please see the our web site https://ruo.mbl.co.jp/je/rip-assay/

SPECIES CROSS REACTIVITY on WB:

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

INTENDED USE: For Research Use Only. Not for use in diagnostic procedures.

Entrez Gene ID: 3184 (Human)

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

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)

RIP-Assay Starter Kit

Each RIP-Assay Starter Kit contains 40 µg of RIP-Certified Antibody and RIP-Assay Kit.

RN001PK	RIP-Assay Starter Kit EIF4E (polyclonal)
RN002PK	RIP-Assay Starter Kit EIF4G1 (polyclonal)
RN003PK	RIP-Assay Starter Kit EIF4G2 (polyclonal)
RN004PK	RIP-Assay Starter Kit ELAVL1/HuR (polyclonal)
RN005PK	RIP-Assay Starter Kit ELAVL2/HuB (polyclonal)
RN006PK	RIP-Assay Starter Kit ELAVL3/HuC (polyclonal)
RN007PK	RIP-Assay Starter Kit IGF2BP1/IMP1 (polyclonal)
RN008PK	RIP-Assay Starter Kit IGF2BP2/IMP2 (polyclonal)
RN009PK	RIP-Assay Starter Kit IGF2BP3/IMP3 (polyclonal)
RN010PK	RIP-Assay Starter Kit MSI1/Musashi1 (polyclonal)
RN011PK	RIP-Assay Starter Kit PTBP1 (polyclonal)
RN012PK	RIP-Assay Starter Kit STAU1 (polyclonal)
RN013PK	RIP-Assay Starter Kit STAU2 (polyclonal)
RN014PK	RIP-Assay Starter Kit TIA1 (polyclonal)
RN015PK	RIP-Assay Starter Kit YBX1 (polyclonal)

RBP Antibody

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

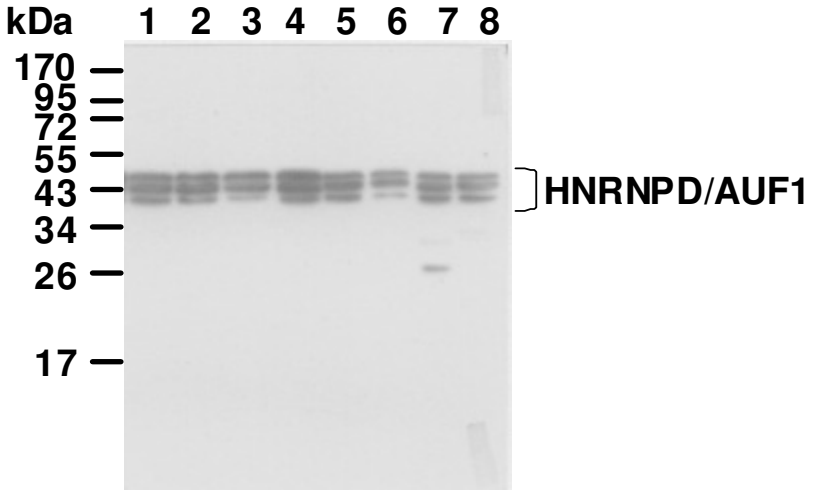
RN023PW	Anti-PABPN1 (polyclonal)
RN028PW	Anti-EIF2C1/AGO1 (polyclonal)
RN029PW	Anti-EIF2C2/AGO2 (polyclonal)
RN030PW	Anti-DICER1 (polyclonal)
RN031PW	Anti-ZFP36 (polyclonal)
RN034PW	Anti-CUGBP1 (polyclonal)
RN035PW	Anti-CUGBP2 (polyclonal)
RN036PW	Anti-ACO1/IRP1 (polyclonal)
RN039PW	Anti-CPEB2 (polyclonal)
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)
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 seconds).
2) Boil the samples for 2 minutes 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 hour 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 hour at room temperature, or overnight at 4°C.
5) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggested in the **APPLICATIONS** for 1 hour 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 minutes x 3 times).
7) Incubate the membrane with the 1:5,000 HRP-conjugated anti-rabbit IgG (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
8) Wash the membrane with PBS-T (10 minutes x 3 times).
9) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. 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 minute. 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, CHO)

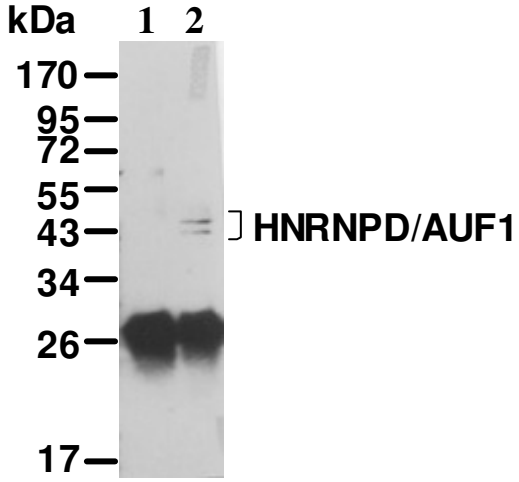


Western blot analysis of HNRNPD/AUF1 in 293T (1), HeLa (2), Jurkat (3), K562 (4), NIH/3T3 (5), WR19L (6), Rat1 (7) and CHO (8) using RN060PW.

Immunoprecipitation

1) Wash 4 x 10^7 cells 2 times with PBS and resuspend them with 1 mL of ice-cold Lysis (150 mM NaCl, 20 mM Tris-HCl, pH 8.0, 0.1% NP-40, 10 mM EDTA) containing appropriate protease inhibitors. Vortex for 10 seconds, then leave on ice for 10 minutes.
2) Centrifuge the tube at 12,000 x g for 5 minutes at 4°C and transfer the supernatant to another tube.
3) Add 40 µL of 50% protein A agarose beads slurry resuspended in Lysis Buffer into the supernatant. Incubate it at 4°C with rotating for 1 hour.
4) Centrifuge the tube at 2,000 x g for 1 minute 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 (RIP-Assay Kit) or anti-HNRNPD/AUF1 antibody at the amount of suggested in the **APPLICATIONS,** and then add 1 mL of Wash Buffer into each tube. Incubate with gently agitation for 1 hour at 4°C.
6) Wash the beads once with ice-cold Lysis Buffer (centrifuge the tube at 2,000 x g for 1 minute). 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 hour at 4°C.
8) Wash the beads 4 times with Wash Buffer (centrifuge the tube at 2,000 x g for 1 minute).
9) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 20 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
10) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour 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.
11) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
12) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggested in the **APPLICATIONS** for 1 hour 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] (10 minutes x 3 times).
14) 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 hour at room temperature.
15) Wash the membrane with PBS-T (10 minutes x 3 times).
16) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. 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 3 minute. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; 293T)



Immunoprecipitation of HNRNPD/AUF1 from 293T with normal rabbit IgG (1) or RN060PW (2).

After immunoprecipitated with antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with RN060PW.