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

A JSR Life Sciences Company

RiboCluster Profiler™

RBP Antibody

Anti-HNRNPC pAb

Code No.: RN052PW

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	1:5,000 for chemiluminescence detection system
Immunoprecipitation	5 µL/500 µL of cell extract from 5 x 10^6 cells

For more information, please visit our web site at https://ruo.mbl.co.jp/.

SPECIES CROSS REACTIVITY on WB:

Species	Human	Mouse	Rat	Hamster
Cells	293T, HeLa, K562	NIH/3T3, WR19L	Not tested	Not tested
Reactivity	+	+		

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Entrez Gene ID: 3183 (Human)

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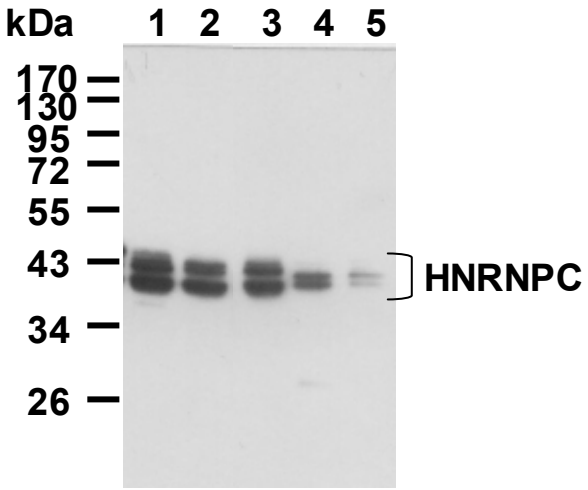
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

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% methanol). 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 1% skimmed milk (in PBS, pH 7.2) 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 1:10,000 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 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; 293T, HeLa, K562, NIH/3T3, WR19L)

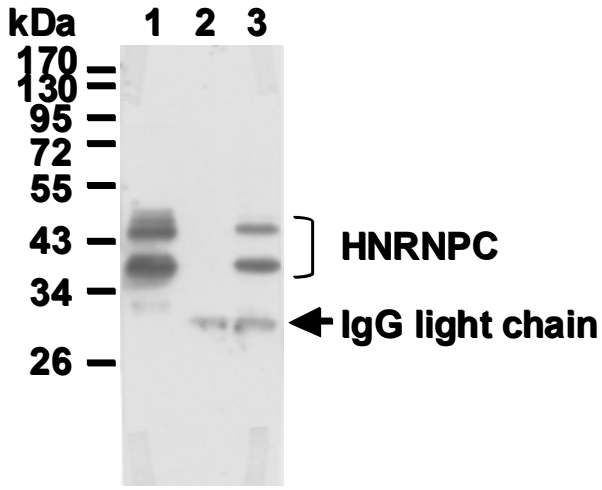


Western blotting analysis of HNRNPC in 293T (1), HeLa (2), K562 (3), NIH/3T3 (4) and WR19L (5) using RN052PW.

Immunoprecipitation

1) Wash 1 x 10^7 cells twice 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. 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-HNRNPC 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 hours 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% methanol). 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 1% skimmed milk (in PBS, pH 7.2) 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 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; 293T)



Immunoprecipitation of HNRNPC from 293T with normal rabbit IgG (2) or RN052PW (3).

After immunoprecipitated with antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with RN052PW.

Lane 1 is the input sample.