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

MBL
A JSR Life Sciences Company

RiboCluster Profiler™

RIP-Certified Antibody

Anti-HNRNPK pAb

Code No.	Quantity	Concentration	Form
RN019P	200 µL	1 mg/mL	Affinity Purified

BACKGROUND: The heterogeneous nuclear ribonucleoprotein K (hnRNP K), is an RNA-binding protein containing 3 KH RNA-binding domains. It binds to both RNA and DNA and is a component of the hnRNP complex. hnRNP K is associated with pre-mRNA and appears to influence the processing of pre-mRNAs and metabolism of mRNA. hnRNP K is predominantly localized at the nucleus and shuttles between the nucleus and the cytoplasm. Therefore, hnRNP K is thought to be involved in the transport of mRNA. Phosphorylation of hnRNP K leads to its cytoplasmic accumulation and is important for cell migration and metastasis. In the cytoplasm, hnRNP K functions as a translational regulator of specific mRNAs such as c-myc mRNA, 16L2 mRNA, and r15-LOX mRNA. Further, it specifically associates with c-src kinase, leading to c-src activation and hnRNP K phosphorylation, which affect the binding of hnRNP K to RNA.

RIP-CERTIFIED ANTIBODY:
Posttranscriptional regulation of gene expression is a ribonucleoprotein-driven process, which involves RNA binding proteins (RBPs) and non-coding RNAs that affect splicing, nuclear export, subcellular localization, mRNA decay and translation. The RNP Immunoprecipitation-Chip (RIP-Chip), RIP-Seq and RIP-RTPCR allow the identification of multiple RNA targets of RBPs globally and within the context of a cell extract. Antibodies specific to the RNA binding protein of interest are used to co-immunoprecipitate the RNA binding protein and the associated subset of mRNAs. The mRNA content is interrogated using standard microarray or sequencing technology. RIP-Certified Antibody is validated for use in RNP Immunoprecipitation (RIP) in conjunction with the RIP-Assay Kit distributed from MBL. Its ability to immunoprecipitate mRNAs and RBPs complex was confirmed by quantitative and qualitative analysis on NanoDrop, Bioanalyzer and RT-PCR or microarray.

SOURCE: This antibody was purified from rabbit serum by affinity column chromatography. The rabbit was immunized with KLH conjugated synthetic peptide, corresponding to C-terminus of human HNRNPK.

FORMULATION: 200 µL volume of 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.

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

REACTIVITY: This antibody reacts with human HNRNPK on Western blotting, Immunoprecipitation and RNP Immunoprecipitation.

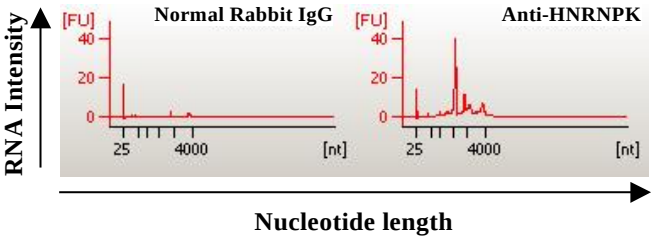
APPLICATIONS:
RNP Immunoprecipitation; 15 µg/500 µL of cell extract from 1.5 x 10^7 cells
Western blotting; 1 µg/mL
Immunoprecipitation; 5 µg/500 µL of cell extract from 5 x 10^6 cells
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested*
*It is reported that this antibody can be used in the reference number 1).
Flow cytometry; Not tested

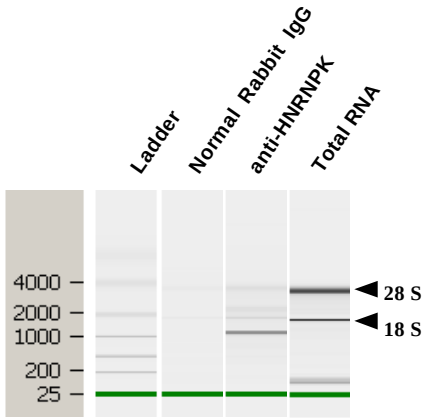
Detailed procedures are provided in the following **PROTOCOLS**.

REFERENCES:
1) Folci, A., *et al.*, *J. Neurosci.* **34**, 9088-9095 (2014) [WB, IC]
2) Adolph, D., *et al.*, *Mol. Cell. Biol.* **27**, 1758-1770 (2007)
3) Inoue, A., *et al.*, *PNAS* **104**, 8983-8988 (2007)
4) Hsieh, T. Y., *et al.*, *J. Biol. Chem.* **273**, 17651-17659 (1998)

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster
Cells	293T, HeLa, K562, Jurkat	NIH/3T3, WR19L	Rat1	Not Tested
Reactivity on WB	+	+	+	





Analysis of isolated RNA with Bioanalyzer.

Average of the RNA Quantity (n=2)	
Antibody	RNA (ng)
Normal Rabbit IgG	74.0
anti-HNRNPK	643.5
Total RNA	228835.0

PROTOCOLS:

RNP Immunoprecipitation

Some buffers and reagents are included in the RIP-Assay Kit (code. RN1001). Please also refer to the protocol packaged in the RIP-Assay Kit.

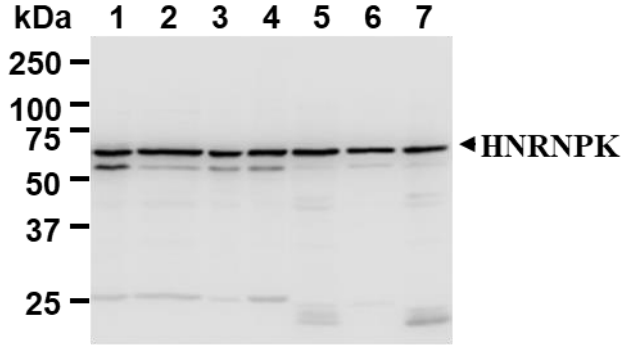
[Material Preparation]

1. Lysis Buffer (+)
   Before using the Lysis Buffer, protease inhibitors, RNase inhibitors, and DTT are added to the Lysis Buffer at the appropriate concentration.
2. Wash Buffer (+)
   Before using the Wash Buffer, DTT is added to the Wash Buffer at the appropriate concentration.

Protocol

1) Wash 1.5 x 10^7 cells 4 times with PBS and resuspend them with 500 µL of ice-cold Lysis Buffer (+) containing appropriate protease inhibitors, RNase inhibitors, and DTT. Vortex thoroughly, then incubate it 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 25 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 fresh tube (precleared sample).
5) Mix 25 µL of 50% protein A agarose beads slurry resuspended in nuclease-free PBS with Normal Rabbit IgG (RIP-Assay Kit) or Anti-HNRNPK pAb (RN019P) at the concentration suggested in the **APPLICATIONS,** and then add 1 mL of Wash buffer (+) into each tube. Incubate with gentle agitation for 1 hour at 4°C.
6) Centrifuge the tube at 2,000 x g for 1 minute, and carefully discard the supernatant using a pipettor without disturbing the beads.
7) Resuspend the beads with ice-cold Lysis Buffer (+).
8) Centrifuge the tube at 2,000 x g for 1 minute, and carefully discard the supernatant.
9) Add 500 µL of cell lysate (precleared sample of step 4), then incubate with gentle agitation for 3 hours at 4°C.
10) Centrifuge the tube at 2,000 x g for 1 minute, and carefully discard the supernatant.
11) Resuspend the beads with Wash Buffer (+).
12) Centrifuge the tube at 2,000 x g for 1 minute, and carefully discard the supernatant.
13) Repeat steps 11)-12) 4 times.
14) Add 400 µL of Master mix solution (Solution I: Solution II = 10 µL: 390 µL). Vortex thoroughly, then spin-down.
15) Add 250 µL of Solution III. Vortex thoroughly.
16) Centrifuge the tube at 2,000 x g for 2 minutes.
17) Transfer the supernatant to the fresh tube containing 2 µL of Solution IV.
18) Add 600 µL of ice-cold 2-propanol and place at -20°C for 20 minutes. Centrifuge the tube at 12,000 x g for 10 minutes.
19) Wash the pellet 2 times with 0.5 mL of ice-cold 70% Ethanol and dry up the pellet for 5-15 minutes.
20) Dissolve the pellets in nuclease-free water.
21) RNA was quantified with NanoDrop (Thermo Fisher Scientific Inc.) and the RNA quality was analyzed with Bioanalyzer (Agilent Technologies, Inc.).

(Positive control for RNP Immunoprecipitation; K562)

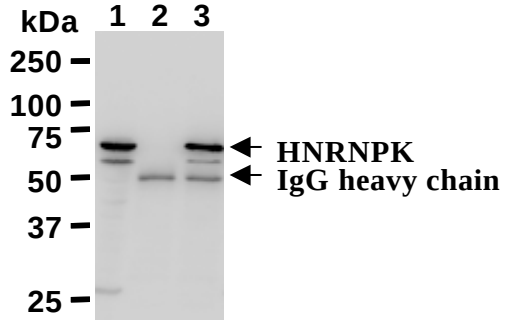


Western blotting analysis of HNRNPK expression in 293T (1), HeLa (2), Jurkat (3), K562 (4), NIH/3T3 (5), WR19L (6) and Rat1 (7) using RN019P.

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.
2) Boil the samples for 2 minutes and centrifuge. Load 10 µL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out 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 manufacturer's manual for precise transfer procedure.
4) To reduce nonspecific binding, soak the membrane in 10% 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] (5 minutes x 3 times).
7) Incubate the membrane with the 1:10,000 Anti-IgG (Rabbit) pAb-HRP (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 (5 minutes x 3 times).
9) Wipe excess buffer off the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute.
10) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
11) The detection was performed with LAS-4000 (FUJIFILM).

(Positive controls for Western blotting; 293T, HeLa, K562, Jurkat, NIH3T3, WR19L and Rat1)



Immunoprecipitation of HNRNPK from K562 with normal rabbit IgG (2) or RN019P (3). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with RN019P. Lane 1 is the input sample.*

Immunoprecipitation

1) Wash cells (approximately 1 x 10^7 cells) 2 times with PBS and resuspend them with 1 mL of ice-cold Lysis buffer (RIP-Assay Kit) containing protease inhibitors and DTT at appropriate concentrations. Vortex thoroughly, then incubate it 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 20 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 20 µL of 50% protein A agarose beads slurry resuspended in PBS with Normal Rabbit IgG (RIP-Assay Kit) or Anti-HNRNPK pAb (RN019P) at the concentration suggested in the **APPLICATIONS,** and then add 1 mL of Wash buffer into each tube. Incubate with gentle agitation for 1 hour at 4°C.
6) Centrifuge the tube at 2,000 x g for 1 minute, and carefully discard the supernatant using a pipettor without disturbing the beads.
7) Resuspend the beads with ice-cold Lysis Buffer (+).
8) Centrifuge the tube at 2,000 x g for 1 minute, and carefully discard the supernatant.
9) Add 500 µL of cell lysate (precleared sample of step 4), then incubate with gentle agitation for 3 hours at 4°C
10) Centrifuge the tube at 2,000 x g for 1 minute, and carefully discard the supernatant.
11) Resuspend the beads with Wash Buffer (+).
12) Centrifuge the tube at 2,000 x g for 1 minute, and carefully discard the supernatant.
13) Repeat steps 11)-12) 4 times
14) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 20 µL/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; K562)

RELATED PRODUCTS:

Please visit website at http://ruo.mbl.co.jp/je/rip-assay/.