

RiboCluster Profiler™

RBP Antibody

Anti-DICER1 (Human) pAb

Code No.	Quantity	Concentration	Form
RN030PW	100 µL	1 mg/mL	Affinity Purified

BACKGROUND: Dicer1 is an endonuclease of the ribonuclease III (RNase III) family. It contains a DExH RNA helicase/ATPase domain, a Piwi/Argonaute/Zwille domain, a domain of unknown function, two RNase III domains, and a double-stranded RNA (dsRNA) binding domain. Dicer1 processes dsRNAs and pre-microRNAs into short RNA fragments of about 21–25 nucleotides in length. It is an initiating factor of the RNA interference pathway and plays a role in microRNA biogenesis and function. Dicer also mediates DNA hypermethylation and gene silencing in human colon cancer cell line.

SOURCE: This antibody was purified from rabbit serum by affinity column chromatography. The rabbit was immunized with KLH conjugated synthetic peptide, corresponding to internal region of human DICER1.

FORMULATION: 100 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.

REACTIVITY: This antibody reacts with human DICER1 on Western blotting and Immunoprecipitation.

APPLICATIONS:

Western blotting: 1 µg/mL for chemiluminescence detection system

Immunoprecipitation: 5 µg/500 µL of cell extract from 5 x 10⁶ cells

Immunohistochemistry: Not tested

Immunocytochemistry: Not tested

Flow cytometry: Not tested

RNP Immunoprecipitation: Not tested

Detailed procedure is provided in the following **PROTOCOLS**.

INTENDED USE:

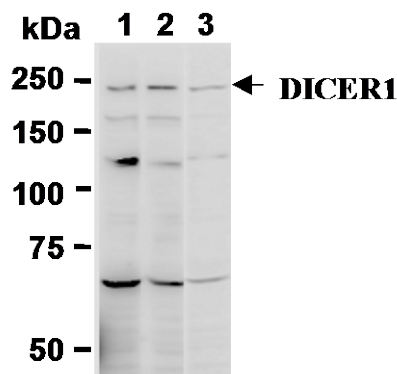
For Research Use Only. Not for use in diagnostic procedures.

REFERENCES:

- 1) Ting, A. H., *et al.*, *Cancer Res.* **68**, 2570-2575 (2008)
- 2) MacLae, I. J., *et al.*, *Science* **311**, 195-198 (2006)
- 3) Zhang, H., *et al.*, *Cell* **118**, 57-68 (2004)

SPECIES CROSS REACTIVITY:

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



Western blot analysis of DICER1 expression in 293T (1), K562 (2) and Jurkat (3) using RN030PW.

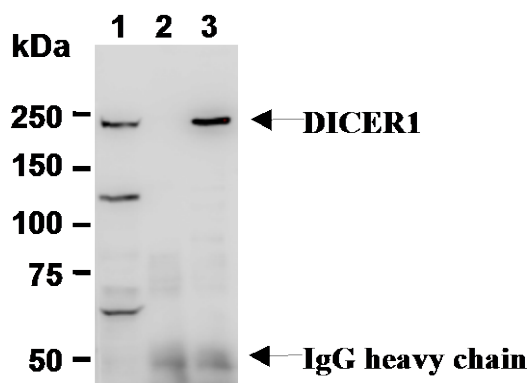
PROTOCOLS:

SDS-PAGE & Western Blotting

- 1) Wash 1 x 10⁷ 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/cm² for 1 hour 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 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 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] (5 minutes x 3 times).
- 7) Incubate the membrane with 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.
- Wash the membrane with PBS-T (5 minutes x 3 times).
 - Wipe excess buffer off the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute.
 - Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
 - The detection was performed with LAS-4000 (FUJIFILM).

(Positive controls for Western blotting; 293T, Jurkat and K562)



Immunoprecipitation of DICER1 from 293T with normal rabbit IgG (2) or RN030PW (3). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with RN030PW. Lane 1 is the input sample.

Immunoprecipitation

- Wash cells (approximately 1×10^7 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.
- Centrifuge the tube at 12,000 x g for 5 minutes at 4°C and transfer the supernatant to another tube.
- Add 20 μ L of 50% protein A agarose beads slurry resuspended in Lysis Buffer into the supernatant. Incubate it at 4°C with rotating for 1 hour.
- Centrifuge the tube at 2,000 x g for 1 minute at 4°C and transfer the supernatant to another tube (precleared sample).
- Mix 20 μ L of 50% protein A agarose beads slurry resuspended in PBS with Normal Rabbit IgG (RIP-Assay Kit) or Anti-DICER1 (Human) pAb (RN030PW) at the amount as suggested in the **APPLICATIONS**, and then add 1 mL of Wash Buffer (RIP-Assay Kit) into each tube. Incubate with gentle agitation for 1 hour at 4°C.
- 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.
- Add 500 μ L of cell lysate (precleared sample of step 4), then incubate with gentle agitation for 3 hours at 4°C.
- Wash the beads 4 times with Wash Buffer (centrifuge the

tube at 2,000 x g for 1 minute).

- Resuspend the beads in 20 μ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 20 μ L/lane for the SDS-PAGE analysis. (See **SDS-PAGE & Western blotting.**)

(Positive control for Immunoprecipitation; 293T)

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 (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 |
| RN011P | Anti-PTBP1 (Human) pAb |
| RN022P | Anti-PABPC4 pAb |
| RN024P | Anti-PCBP1 pAb |
| RN025P | Anti-PCBP2 pAb |
| RN033P | Anti-TNRC6A (GW182) (Human) pAb |
| RN001M | Anti-IGF2BP1 (IMP1) mAb |
| 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

- | | |
|---------|-------------------------------|
| RN028PW | Anti-EIF2C1 (AGO1) pAb |
| RN029PW | Anti-EIF2C2 (AGO2) pAb |
| RN034PW | Anti-CUGBP1 pAb |
| RN057PW | Anti-TARBP1 pAb |
| RN058PW | Anti-TARBP2 pAb |
| RN062PW | Anti-DGCR8 pAb |
| RN065PW | Anti-KHSRP pAb |
| RN074PW | Anti-SSA (La) pAb |
| RN114PW | Anti-HNRNPA1 pAb |
| RN115PW | Anti-LIN28B (Human) pAb |
| RN116PW | Anti-DDX39B (UAP56) pAb |
| RN127PW | Anti-NSUN2 (Human) pAb |
| RN129PW | Anti-DDX6 (RCK/p54) pAb |
| RN002MW | Anti-CUGBP1 mAb (3B1) |
| RN008MW | Anti-ELAVL1 (HuR) mAb (C54-6) |
| RN010MW | Anti-PIWIL1 (MIWI) mAb (2D9) |

For the latest information of RiboCluster Profiler™, Please visit website at <http://ruo.mbl.co.jp/je/rip-assay/>.

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