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Not for use in diagnostic procedures.

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RiboCluster Profiler™ **RIP-Certified Antibody**

Anti-EIF2C2 (AGO2) (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
RN003M	1B1-E2H5	Mouse IgG2a λ	200 µL	1 mg/mL

BACKGROUND: Eukaryotic translation initiation factor 2C, subunit 2 (EIF2C2; AGO2), is a member of the argonaute protein family. It is a core component of RNA-induced silencing complex (RISC). The EIF2C2 belonging to the human argonaute protein family possesses endonuclease activity and functions as a slicer in gene silencing pathways. It recognizes and cleaves target mRNA by RNA interference. In small interference RNA (siRNA) pathway, EIF2C2 forms a complex with EIF2C1 and siRNA, and in microRNA (miRNA) pathway, it binds EIF2C3 to miRNA. EIF2C2 expression is controlled by epidermal growth factor receptor and mitogen-activated protein kinase signaling in human breast cancer cell line.

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 RNAs. The RNA 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 *for microRNA* distributed from MBL. Its ability to immunoprecipitate RNAs and RBPs complex was confirmed by quantitative and qualitative analysis on NanoDrop, Bioanalyzer and RT-PCR or microarray.

SOURCE: This antibody was purified from hybridoma (clone 1B1-E2H5) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse lymphocyte immunized with human EIF2C2 fusion protein corresponding to N-terminal regions.

FORMULATION: 200 µg IgG in 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.

REACTIVITY: This antibody reacts with human EIF2C2 (~93.6 kDa) on Western blotting and Immunoprecipitation. Other species cross reactivity is confirmed by Western blotting.

INTENDED USE:
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APPLICATIONS:
RNP Immunoprecipitation; 15 µg/500 µL of cell extract from 8.5 x 10^6 cells
Western blotting; 1-4 µg/mL for chemiluminescence detection system
Immunoprecipitation; 1.5 µg/500 µL of cell extract from 1.5x 10^6 cells
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

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

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13) Azuma-Mukai, A., *et al., PNAS* **105**, 7964-7969 (2008)
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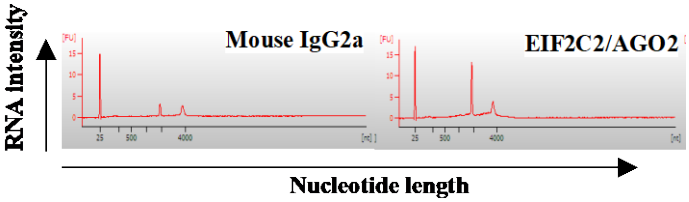
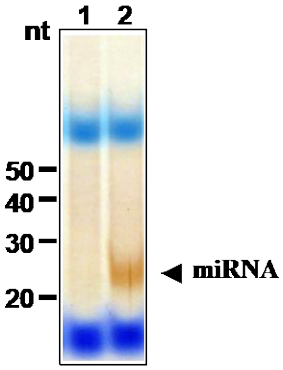




Analysis of isolated small RNA (including miRNA) from Jurkat by silver staining following denaturing PAGE.
Lane1: Mouse IgG2a Isotype Control
Lane2: RN003M.

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

PROTOCOLS:

RNP Immunoprecipitation

Some buffers and reagents are included in the RIP-Assay Kit *for microRNA* (code. RN1005). Please also refer to the protocol packaged in the RIP-Assay Kit *for microRNA*.

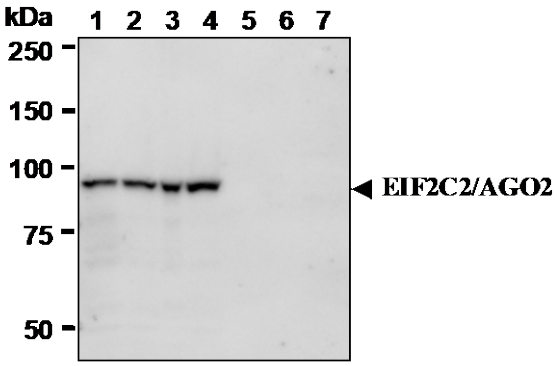
[Material Preparation]

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

Protocol (RNA isolation: Separation method)

1) Wash 8.5 x 10^6 cells 4 times with PBS and resuspend them with 500 µL of ice-cold mi-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 30 µL of 50% protein A agarose beads slurry resuspended in mi-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 30 µL of 50% protein A agarose beads slurry resuspended in nuclease-free PBS with Mouse IgG2a (isotype control) (MBL; code no. M076-3) or Anti-EIF2C2 (AGO2) (Human) mAb (RN003M) at the amount as suggested in the **APPLICATIONS,** and then add 1 mL of mi-Wash Buffer (+) into each tube. Incubate with gentle agitation for 1 hour at 4°C.
6) Wash the beads once with ice-cold mi-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 mi-Wash Buffer (+) (centrifuge the tube at 2,000 x g for 1 minute).
9) Add 250 µL of Master mix solution (mi-Solution I: mi-Solution II = 10 µL: 240 µL). Vortex thoroughly, then spin-down.
10) Add 150 µL of mi-Solution III. Vortex thoroughly.
11) Centrifuge the tube at 2,000 x g for 2 minutes.
12) Transfer the supernatant to the fresh tube containing 2 µL of mi-Solution IV.
13) Add 300 µL of ice-cold 2-propanol, vortex for 10 seconds. Place at -20°C for 20 minutes. Centrifuge the tube at 12,000 x g for 10 minutes.
14) Transfer the supernatant, which contains small RNAs, to the tube containing 2 µL of mi-Solution IV. Isolation method for small RNAs from the supernatant is described in the following step 15. In case of purification for large RNAs in the pellet, skip to step 16.
15) Add 500 µL of ice-cold 2-propanol, vortex for 10 seconds, then place at -20°C for 20 minutes. Centrifuge the tube at 12,000 x g for 10 minutes.
16) Wash the pellet 2 times with 0.5 mL of ice-cold 70% ethanol and dry up the pellet for 5-15 minutes.
17) Dissolve the pellets in nuclease-free water.

(Positive control for RNP Immunoprecipitation; Jurkat)



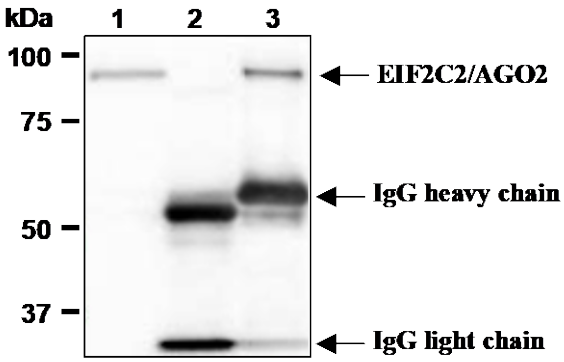
Western blot analysis of EIF2C2/AGO2 expression in 293T (1), HeLa (2), K562 (3), Jurkat (4), NIH/3T3 (5), WR19L (6) and Rat1 (7) using RN003M.

SDS-PAGE & Western Blotting

1) Wash cells (approximately 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% MeOH). See the manufacture'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 1:5,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) 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).



Immunoprecipitation of EIF2C2/AGO2 from Jurkat with mouse IgG2a isotype control (2) or RN003M (3). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with RN003M. Lane 1 is the input sample.

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

Immunoprecipitation

1) Wash cells (approximately 1.5 x 10^6 cells) 2 times with PBS and resuspend them with 1 mL of ice-cold mi-Lysis buffer (+) (RIP-Assay Kit *for microRNA*) 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 fresh tube.
3) Add 25 µL of 50% protein A agarose beads slurry resuspended in mi-Lysis Buffer (+) into the supernatant. Incubate it at 4ºC 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 PBS with Mouse IgG2a (isotype control) (MBL; code no. M076-3) or Anti-EIF2C2 (AGO2) (Human) mAb (RN003M) at the amount as suggested in the **APPLICATIONS,** and then add 1 mL of mi-Wash buffer (+) into each tube. Incubate with gentle agitation for 1 hour at 4°C.
6) Wash the beads once with ice-cold mi-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 mi-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/lane for the SDS-PAGE analysis.
   (See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; Jurkat)

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