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Not for use in diagnostic procedures.

MBL
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RiboCluster Profiler™ **RIP-Certified Antibody**

Anti-TNRC6A (GW182) (Human) pAb

Code No.	Quantity	Concentration	Form
RN033P	200 µL	1 mg/mL	Affinity Purified

BACKGROUND: The trinucleotide repeat containing 6A (TNRC6A), also known as GW182, was so named because of its molecular weight and the presence of multiple glycine (G)–tryptophan (W) amino acid pairs in the N-terminal region that is required for interaction with Argonaute proteins. GW182 is a critical component of cytoplasmic processing bodies (P-bodies), which have been shown to function in mRNA degradation, storage, and miRNA-mediated gene silencing. The interaction of GW182 with Argonautes is required for P-body localization and may be critical for miRNA-mediated translational repression, because it induces the formation of the complexes containing miRNA–target mRNA. Further, the phosphorylated form of human TNRC6A has been shown to play a role in miRNA mediated gene silencing.

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 rabbit serum by affinity column chromatography. The rabbit was immunized with KLH conjugated synthetic peptide, corresponding to internal region of human TNRC6A.

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.

REACTIVITY: This antibody reacts with human TNRC6A on Western blotting, Immunoprecipitation and RNP Immunoprecipitation.

INTENDED USE:
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APPLICATIONS:
RNP Immunoprecipitation; 15-25 µg/500 µL of cell extract from 1.3 x 10^7 cells
Western blotting; 1 µg/mL
Immunoprecipitation; 5 µg/500 µL of cell extract from 1.0 x 10^7 cells
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested*
* It is reported that this antibody can be used in Immunocytochemistry in the reference number 2).
Flow cytometry; Not tested

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

REFERENCES:
1) Fukao, A., *et al*., *Mol. Cell* **56**, 79-89 (2014) [WB]
2) Otsuka, M., *et al*., *PLoS One* **6**, e24359 (2011) [WB, IC]
3) Eulalio, A., *et al*., *RNA* **15**, 1433-1442 (2009)
4) Eulalio, A., *et al*., *Nat. Struct. Mol. Biol.* **15**, 346-353 (2008)

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster
Cells	293T, HeLa, Jurkat	NIH3T3	Rat1	Not tested
Reactivity on WB	+	-	-	

RELATED PRODUCTS:
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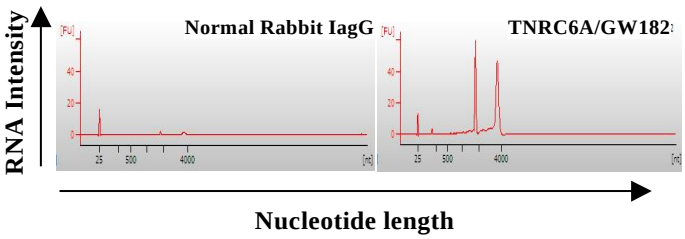
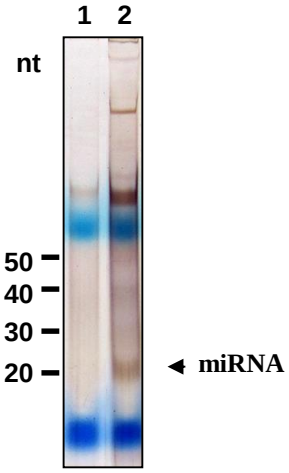
For the latest information of RiboCluster Profiler™, please visit our website at https://ruo.mbl.co.jp/je/rip-assay/.

Average of the RNA Quantity (n=2)	
Antibody	RNA (ng)
Normal Rabbit IgG	71.4
anti-TNRC6A/GW182 pAb	1144.5
Total RNA	262520.0





Analysis of isolated small RNA (including miRNA) from K562 by silver staining following denaturing PAGE.
Lane 1: Normal Rabbit IgG
Lane 2: RN033P.

PROTOCOLS:

RNP Immunoprecipitation

Some buffers and reagents are included in the RIP-Assay Kit *for microRNA* (MBL, code no. RN1005). Please also refer to the protocol packaged in the RIP-Assay Kit *for microRNA*.

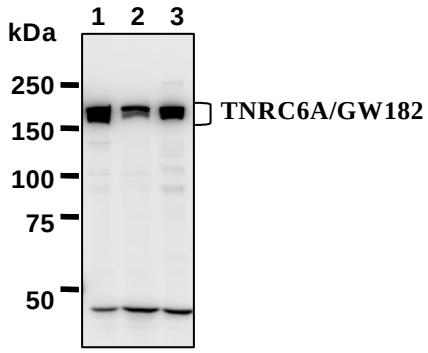
[Material Preparation]

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 1.3 x 10^7 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 Normal Rabbit IgG (RIP-Assay Kit *for microRNA*) or Anti-TNRC6A (GW182) (Human) pAb (MBL, code no. RN033P) at the concentration 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) 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 mi-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 ice-cold mi-Wash Buffer (+).
12) Centrifuge the tube at 2,000 x g for 1 minute, and carefully discard the supernatant.
13) Repeat steps 11)-12) 3 times.
14) Add 250 µL of Master mix solution (mi-Solution I: mi-Solution II = 10 µL: 240 µL). Vortex thoroughly, then spin-down.
15) Add 150 µL of mi-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 mi-Solution IV.
18) 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.
19) 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 20. In case of purification for large RNAs in the pellet, skip to step 21).
20) 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.
21) Wash the pellet twice with 0.5 mL of ice-cold 70% ethanol and dry up the pellet for 5-15 minutes.
22) Dissolve the pellets in nuclease-free water.

(Positive control for RNP Immunoprecipitation; K562)

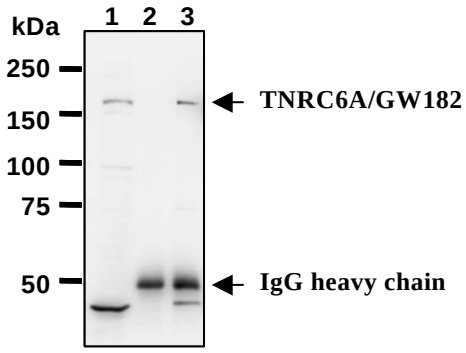


Western blot analysis of TNRC6A expression in 293T (1), HeLa (2) and Jurkat (3) using RN033P.

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).
7) Incubate the membrane with 1:10,000 Anti-IgG (H+L chain) (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).
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 and Jurkat)



Immunoprecipitation of TNRC6A from HeLa with normal rabbit IgG (2) or RN033P (3). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with RN033P. Lane 1 is the input sample.*

Immunoprecipitation

1) Wash cells (approximately 1.0 x 10^7 cells) twice 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 20 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 20 µL of 50% protein A agarose beads slurry resuspended in PBS with Normal Rabbit IgG (RIP-Assay Kit *for microRNA*) or Anti-TNRC6A (GW182) (Human) pAb (MBL, code no. RN033P) at the concentration as suggested in **APPLICATIONS,** and then add 1 mL of mi-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 mi-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 ice-cold mi-Wash Buffer (+).
12) Centrifuge the tube at 2,000 x g for 1 minute, and carefully discard the supernatant.
13) Repeat steps 11)-12) 3 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; HeLa)