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

MBL
A JSR Life Sciences Company

RiboCluster Profiler™ **RIP-Certified Antibody**

Anti-TIA1 pAb

Code No.	Quantity	Concentration	Form
RN014P	200 µL	1 mg/mL	Affinity Purified

BACKGROUND: T-cell intracellular antigen-1 (TIA1), an apoptosis promoting factor, is a RNA binding protein which associates with subsets of mRNAs bearing U-rich sequences in their 3'-UTRs and shuttles between nucleus and cytoplasm. TIA1 possesses three RNA recognition motifs (RRMs). RRM2 and the first half of the auxiliary region are necessary for nuclear accumulation whereas RRM3 mediates nuclear export. TIA1 regulates splicing of alternatively spliced pre-mRNAs including Fas, msl-2, FGFR-2, calsitonin/CGRP in nucleus. In cytoplasm, TIA1 regulates translation of various mRNAs, include TNF-α, Cox-2, HMMP-13 and β2-adrenergic receptor, by binding to AU-rich elements (AREs) located in these mRNA 3' untranslated regions (3'UTRs). In addition to the translational silencing, TIA1 plays a role in the cellular response to environmental stress by promoting the assembly of a non-canonical 48S preinitiation complex that is the core component of cytoplasmic stress granules (SG). Like other mRNA turnover and translation regulators such as AUF-1, HuR, KSRP and NF90, TIA1 also associates its cognate mRNA and transcripts of other RBPs.

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, NGSMLPNQPSGYRVAGYETQ corresponding to 367-386 aa.

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 TIA1 (~43 kDa) on Western blotting, Immunoprecipitation and RNP Immunoprecipitation.

APPLICATIONS:
RNP Immunoprecipitation; 15 µg/500 µL of cell extract from 6 x 10^6 cells
Western blotting; 1:1,000
Immunoprecipitation; 5 µg/250 µL of cell extract from 2.5 x 10^6 cells
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

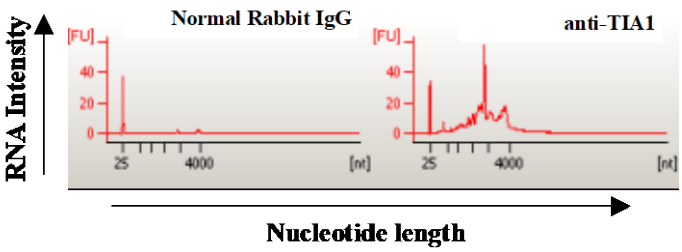
Detailed procedure is provided in the following **PROTOCOLS**.

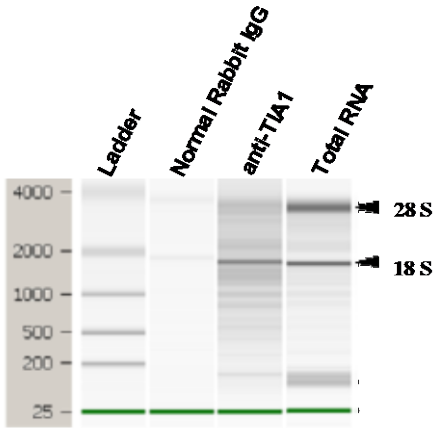
REFERENCES:
1) Pullmann, Jr., R., *et al., Mol. Cell Biol.* **27**, 6265-6278 (2007)
2) Zhang, T., *et al., J. Cell Sci.* **118**, 5453-5463 (2005)
3) Kandasamy, K., *et al., J. Biol. Chem.* **280**, 1931-1943 (2005)

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster
Cells	K562, 293T, HeLa, Jurkat	NIH/3T3, WR19L	Rat1	CHO
Reactivity on WB	+	+	+	+

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





Analysis of isolated RNA with Bioanalyzer.

Average of the RNA Quantity (n=2)	
Antibody	RNA (ng)
Normal Rabbit IgG	53.0
anti-TIA1	515.0
Total RNA	84570.0

ROTOCOLS:

RNP Immunoprecipitation

Some buffer and reagents are included in the RIP-Assay Kit (MBL, code no. RN1001). Please also refer to the protocol packaged in the RIP-Assay Kit.

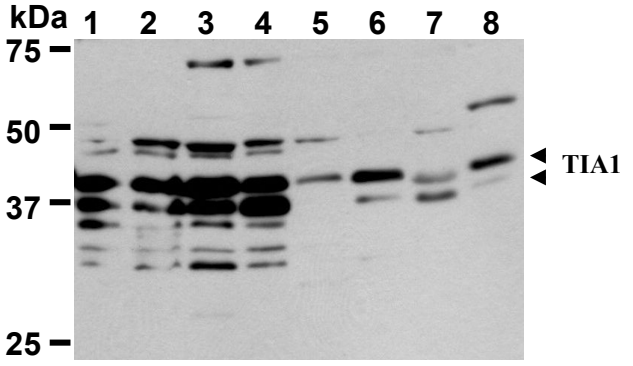
[Material Preparation]

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 6 x 10^6 cells twice with PBS and resuspend them with 500 µL of ice-cold Lysis Buffer (+) containing appropriate protease inhibitors, RNase inhibitors, and DTT. Vortex for 10 seconds. 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 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 tube (precleared sample).
5) Mix both 25 µL of 50% protein A agarose beads slurry resuspended in nuclease-free PBS and Normal Rabbit IgG (RIP-Assay Kit) or Anti-TIA1 pAb (MBL, code no. RN014P) at the amount of 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 using a pipettor without disturbing the beads.
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) 3 times
14) Add 400 µL of Master mix solution (Solution I: Solution II = 10 µL: 390 µL). Vortex for 10 seconds.
15) Add 250 µL of Solution III. Vortex for 10 seconds.
16) Centrifuge the tube at 2,000 x g for 2 minutes.
17) Transfer the supernatant to the 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 twice with 500 µL 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; Jurkat)

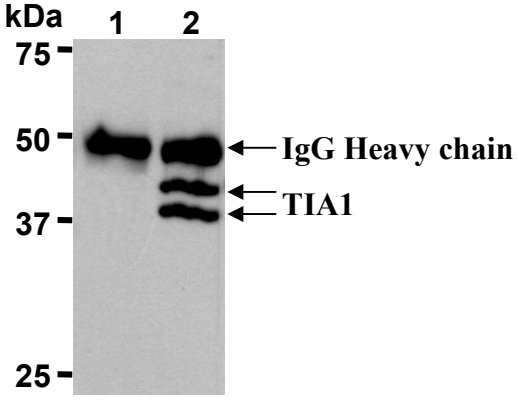


Western blotting analysis of TIA1 expression in K562 (1), 293T (2), HeLa (3), Jurkat (4), NIH/3T3 (5), WR19L (6) Rat1 (7) and CHO (8) using RN014P.

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 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 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 of 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).
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 1 minute. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; K562, 293T, HeLa, Jurkat, NIH/3T3, WR19L, Rat1 and CHO)



Immunoprecipitation of TIA1 from Jurkat with normal rabbit IgG (1) or RN014P (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with RN014P.*

Immunoprecipitation

1) Wash 1 x 10^7 cells twice with PBS and resuspend them with 1 mL of ice-cold Lysis buffer (RIP-Assay Kit) containing appropriate protease inhibitors, then sonicate briefly (up to 10 seconds).
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 both 20 µL of 50% protein A agarose beads slurry resuspended in nuclease-free PBS and Normal Rabbit IgG (RIP-Assay Kit) or Anti-TIA1 pAb (MBL, code no. RN014P) 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) 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 250 µL of cell lysate (precleared sample of step 4), then incubate with gentle agitation for 1 hour at 4°C.
10) Centrifuge the tube at 2,000 x g for 1 minute, and carefully discard the supernatant using a pipettor without disturbing the beads.
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) 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; Jurkat)