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

MBL
A JSR Life Sciences Company

RiboCluster Profiler™

RIP-Certified Antibody

Anti-EIF4E pAb

Code No.	Quantity	Form
RN001P	200 µL	Affinity Purified

BACKGROUND: The eukaryotic initiation factor 4E (eIF4E) is a key regulatory component that anchors the mRNA cap-binding complex (eIF4F) to the 5' end of capped mRNAs. eIF3, the poly (A)-binding protein, and the eIF4 proteins recruit mRNA to the 43S initiation complex to form the 48S initiation complex. The eIF4 proteins consist of eIF4A; RNA helicase (46 kDa), eIF4B; RNA binding and RNA annealing protein (70 kDa), eIF4E (25 kDa); cap binding protein, eIF4H; work with eIF4B to stimulate eIF4A helicase activity (25 kDa), and eIF4G; co-localize all other proteins necessary for mRNA recruitment on the 40S subunit. As a principal initiation factor, eIF4E has the potential to influence expression of every protein in the cell. mRNA subset associated with eIF4E in p19 cell differed from mRNA subset associated with HuB or poly A binding protein, suggested that they reflect distinct functional subset of mRNAs whose expression is regulated differently at the level of translation.

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, MATVEPETTPTPNPPTTEEEKTESNQEVANPEHYIKH corresponding to 1-37 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.

REACTIVITY: This antibody reacts with human EIF4E (~25 kDa) on Western blotting, Immunoprecipitation and RNP Immunoprecipitation.

APPLICATIONS:
RNP Immunoprecipitation; 15 µL/500 µL of cell extract from 4.5 x 10^6 cells
Western blotting; 1:1,000
Immunoprecipitation; 5 µL/250 µL of cell extract from 2.5 x 10^6 cells
Immunohistochemistry; Not tested
Immunocytochemistry; Can be used
Flow cytometry; Not tested

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

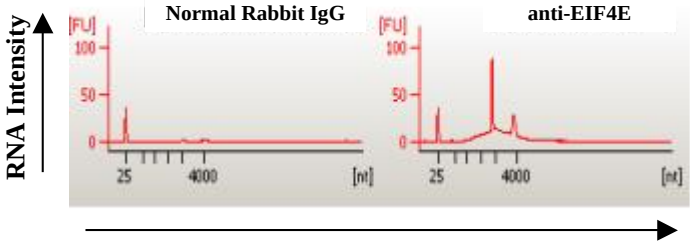
INTENDED USE:
For Research Use Only. Not for use in diagnostic procedures.

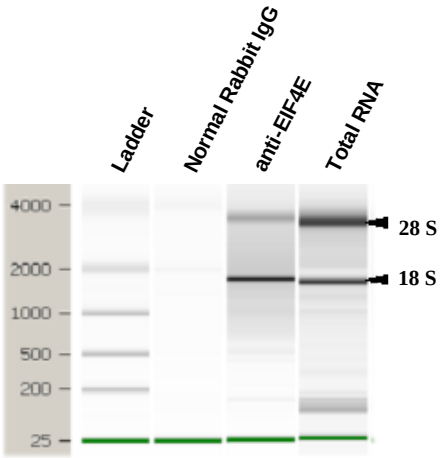
REFERENCES:
1) Culjkovic-Kraljacic, B., *et al*., *Blood* **127**, 858-868 (2016) [RIP]
2) Fukao, A., *et al*., *Mol. Cell* **56**, 79-89 (2014) [WB]
3) Hayman, T. J., *et al., Cancer Res.* **72**, 2362-2372 (2012) [RIP]
4) Thompson, K., *et al., Translational Neuroscience* **1**, 268-278 (2010) [RIP]
5) Rhoads, R. E., *J. Biol. Chem*. **284**, 16711-16715 (2009)
6) Kapp, L. D., and Lorsch, J. R., *Annu. Rev. Biochem* **73**, 657-704 (2004)
7) Tenenbaum, S. A., *et al*., *PNAS* **97**, 14085-14090 (2000)

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster
Cells	MDA-MB-231 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=3)	
Antibody	RNA (ng)
Normal Rabbit IgG	30.0
anti-EIF4E	327.0
Total RNA	72075.0

PROTOCOLS:

RNP Immunoprecipitation

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

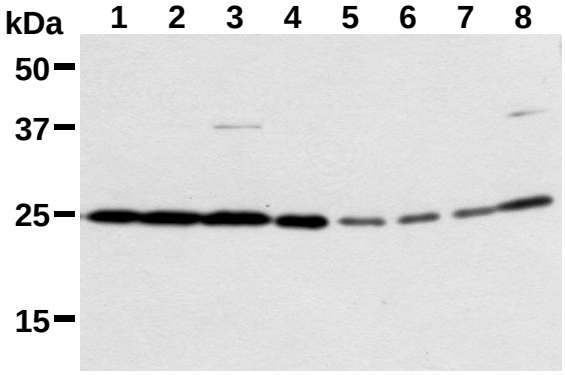
[Material Preparation]

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 4.5 x 10^6 cells 2 times 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-EIF4E pAb (RN001P) 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) 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.
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 Wash Buffer (+) (centrifuge the tube at 2,000 x g for 1 minute).
9) Add 400 µL of Master mix solution (Solution I: Solution II = 10 µL: 390 µL). Vortex for 10 seconds.
10) Add 250 µL of Solution III. Vortex for 10 seconds.
11) Centrifuge the tube at 2,000 x g for 2 minutes.
12) Transfer the supernatant to the tube containing 2 µL of Solution IV.
13) 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.
14) Wash the pellet 2 times with 0.5 mL of ice-cold 70% Ethanol and dry up the pellet for 5-15 minutes.
15) Dissolve the pellets in nuclease-free water.
16) 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; MDA-MB-231)

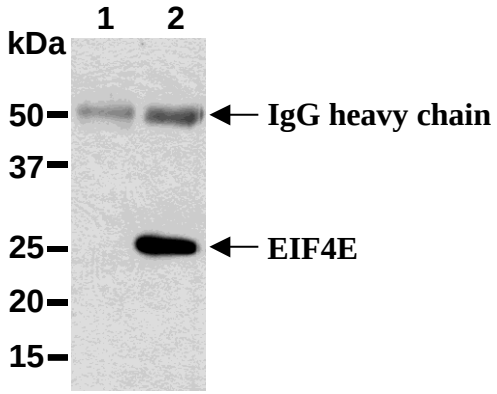


Western blot analysis of EIF4E expression in K562 (1), 293T (2), HeLa (3), Jurkat (4), NIH/3T3 (5), WR19L (6) Rat1 (7) and CHO (8) using RN001P.

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% 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 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 condition.)
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 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 times).
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.



Immunoprecipitation of EIF4E from MDA-MB-231 with normal rabbit IgG (1) or RN001P (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with RN001P.*

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

Immunoprecipitation

1) Wash 1 x 10^7 cells 2 times 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-EIF4E pAb (RN001P) 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) 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.
7) Add 250 µL of cell lysate (precleared sample of step 4), then incubate with gentle agitation for 1 hour at 4°C.
8) Wash the beads 4 times with 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; MDA-MB-231)

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