

**RiboCluster Profiler™**

**RIP-Certified Antibody**

# Anti-eIF4G1 (Human) pAb

**Code No.**  
RN002P

**Quantity**  
200 µL

**Form**  
Affinity Purified

**BACKGROUND:** The eukaryote polypeptide chain initiation factor eIF4G forms complex with the mRNA cap-binding protein eIF4E and the ATP dependent RNA helicase eIF4A, constituting the initiation factor eIF4F complex. The eIF4G plays an important role in the mechanism of translation by tethering between other components of the ribosomal initiation factors. Two forms of eIF4G have been reported as two different gene products, eIF4G1 and 2. The eIF4G1 is a single polypeptide chain of 154~ kDa. The association of eIF4E with eIF4G1 results in the formation of a protease-resistant, 98-residue fragment of eIF4G1. NMR analysis study showed that this residue to be unfolded in the absence of eIF4E and almost completely folded in the presence of eIF4E. It is suggested that the eIF4E binding creates a new binding surface on eIF4G1 that allows for the binding of other factors of the translational machinery. Interaction of PABP with eIF4G1 has been suggested to facilitate the functional association of the 3' end of an mRNA with the 5' end.

## 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-RT-PCR 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, GEELLPESTPIPANLS corresponding to 480-496 aa of human eIF4G1.

**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 eIF4G1 (~175.5 kDa) on Western blotting, Immunoprecipitation and RNP Immunoprecipitation.

## APPLICATIONS:

RNP Immunoprecipitation; 15 µL/500 µL of cell extract from 4.5 x 10<sup>6</sup> cells

Western blotting; 1:1,000

Immunoprecipitation; 5 µL/250 µL of cell extract from 2.5 x 10<sup>6</sup> cells

Immunohistochemistry; Not tested

Immunocytochemistry; Not tested

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:

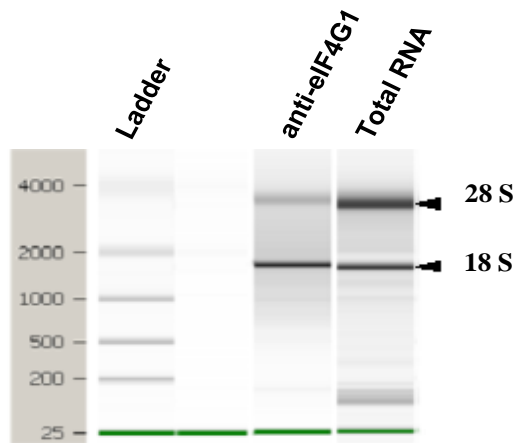
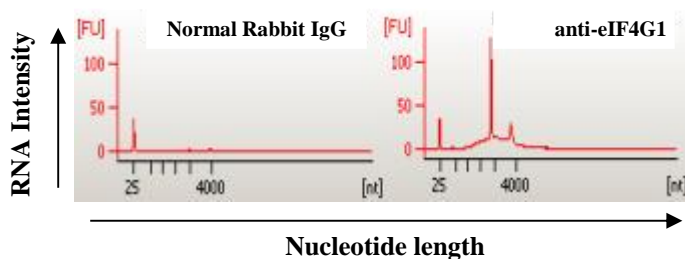
- 1) Gradi, A., *et al.*, *Mol. Cell Biol.* **18**, 334-342 (1998)
- 2) Tarun, Jr. S. Z., *et al.*, *PNAS* **94**, 9046-9051 (1997)
- 3) Yan, R., *et al.*, *J. Biol. Chem.* **267**, 23226-23231 (1992)

## SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster
Cells	MDA-MB-231, K562, 293T, HeLa, Jurkat	NIH/3T3, WR19L	Rat1	CHO
Reactivity on WB	+	-	-	-

## RELATED PRODUCTS:

Please visit our website at <https://ruo.mbl.co.jp/>.



**Analysis of isolated RNA with Bioanalyzer.**

Average of the RNA with Quantity(n=3)	
Antibody	RNA(ng)
Normal Rabbit IgG	29.0
anti-eIF4G1	511.0
Total RNA	72075.0

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

**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.

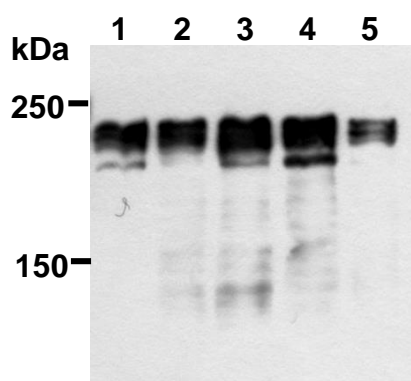
**Protocol**

- 1) Wash  $4.5 \times 10^6$  cells 2 times with PBS and resuspend them with 500  $\mu$ L of ice-cold Lysis Buffer (+) containing appropriate protease inhibitors, RNase inhibitors, and

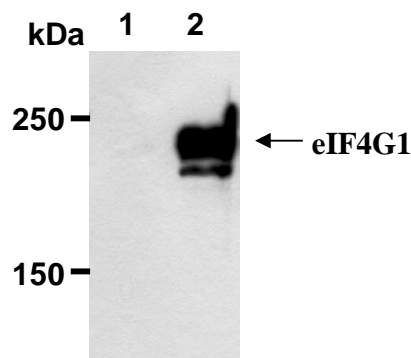
DTT. Vortex for 10 seconds. Leave on ice for 10 minutes.

- 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  $\mu$ 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.
- 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  $\mu$ L of 50% protein A agarose beads slurry resuspended in nuclease-free PBS and Normal Rabbit IgG (RIP-Assay Kit) or Anti-eIF4G1 (Human) pAb (RN002P) 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  $\mu$ L 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) 4 times.
- 14) Add 400  $\mu$ L of Master mix solution (Solution I: Solution II = 10  $\mu$ L: 390  $\mu$ L). Vortex for 10 seconds.
- 15) Add 250  $\mu$ L 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  $\mu$ L of Solution IV.
- 18) Add 600  $\mu$ L 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 2 times with 0.5 mL 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; MDA-MB-231)



**Western blotting analysis of eIF4G1 expression in MDA-MB-231 (1), K562 (2), 293T (3), HeLa (4) and Jurkat (5) using RN002P.**



**Immunoprecipitation of eIF4G1 from MDA-MB-231 with normal rabbit IgG (1) or RN002P (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with RN002P.**

### **SDS-PAGE & Western blotting**

- 1) Wash  $1 \times 10^7$  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  $\mu$ L 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<sup>2</sup> 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 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.

(Positive controls for Western blotting; MDA-MB-231, K562, 293T, HeLa and Jurkat)

### **Immunoprecipitation**

- 1) Wash  $1 \times 10^7$  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  $\mu$ 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.
- 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  $\mu$ L of 50% protein A agarose beads slurry resuspended in nuclease-free PBS and Normal Rabbit IgG (RIP-Assay Kit) or Anti-eIF4G1 (Human) pAb (RN002P) 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 250  $\mu$ L 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) 4 times.
- 14) Resuspend the beads in 20  $\mu$ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 20  $\mu$ L/lane for the SDS-PAGE analysis.

(See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; MDA-MB-231)