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Not for use in diagnostic procedures.

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RiboCluster Profiler™ RIP-Certified Antibody

Anti-ELAVL1 (HuR) mAb

CODE No. RN007M

CLONALITY Monoclonal
CLONE C67-1
ISOTYPE Mouse IgG2a κ
QUANTITY 200 µL

SOURCE Purified IgG from hybridoma supernatant
FORMULATION 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.

APPLICATIONS

RNP immunoprecipitation (RIP)	15 µg/500 µL of cell extract from 1.65 x 10^7 cells
Western blotting	1 µg/mL
Immunoprecipitation	1.5 µg/50 µL of cell extract from 1.65 x 10^6 cells

SPECIES CROSS REACTIVITY on WB

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

Entrez Gene ID 1994 (Human), 15568 (Mouse), 363854 (Rat), 100754131 (Hamster)

REFERENCES

1) Ma, W. J., *et al., J. Biol. Chem*. **271**, 8144-8151 (1996)
2) Fan, X. C., and Steitz, J. A., *PNAS* **95**, 15293-15298 (1998)
3) Fan, X. C., and Steitz, J. A., *EMBO J*, **17**, 3448-3460 (1998)

For more information, please visit our website at **https://ruo.mbl.co.jp/.**

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

RNP immunoprecipitation

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

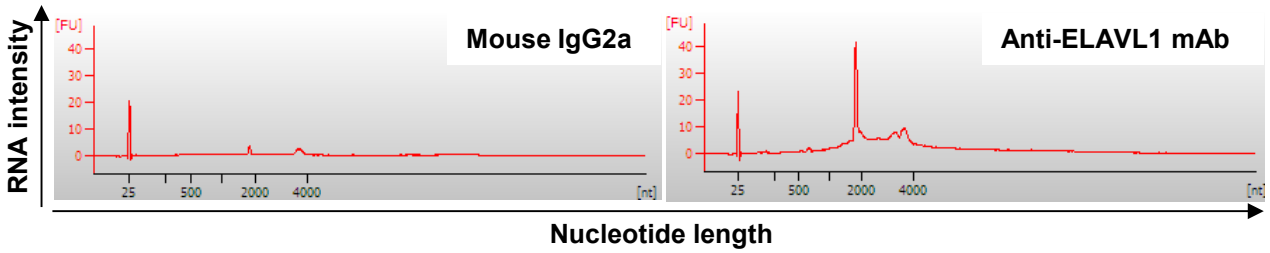
[Material Preparation]

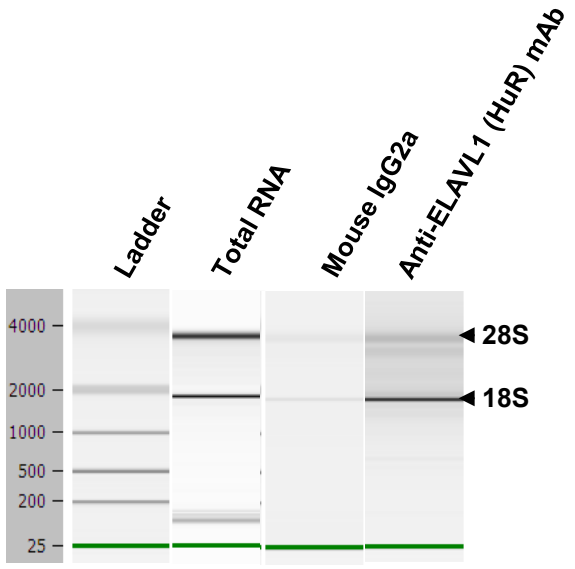
1. Lysis Buffer (+)
   Before using the Lysis Buffer, protease inhibitors, RNase inhibitors, and DTT are added to the Lysis Buffer at the appropriate concentration.
2. Wash Buffer (+)
   Before using the Wash Buffer, DTT is added to the Wash Buffer at the appropriate concentration.
3. Antibody conjugated Protein G beads
   A) Mix 25 µL of 50% protein G agarose beads slurry resuspended in nuclease-free PBS with 1 mL of Wash Buffer (+), and then add Mouse IgG2a (MBL; code no. M076-3) or Anti-ELAVL1 (HuR) mAb at the concentration suggested in the **APPLICATIONS**. Incubate with gentle agitation for 1 hr. at 4°C.
   B) During pre-clear steps (Protocol 3)), wash the beads 1 time with ice-cold Lysis Buffer (+) (centrifuge the tube at 2,000 x g for 1 min.). Carefully discard the supernatant using a pipettor without disturbing the beads and incubate at 4°C until just before use.

[Protocol]

1) Wash the cells (1.65 x 10^7 cells/sample) 4 times with PBS and resuspend them with 500 µL of ice-cold Lysis Buffer (+) containing appropriate protease inhibitors, RNase inhibitors, and DTT. Vortex thoroughly, then incubate on ice for 10 min.
2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
3) Add 25 µL of 50% protein G agarose beads slurry resuspended in Lysis Buffer (+) into the supernatant. Incubate it at 4°C with rotating for 1 hr.
4) Centrifuge the tube at 2,000 x g for 1 min. at 4°C and transfer the supernatant to the tube containing antibody conjugated beads, then incubate with gentle agitation for 3 hr. at 4°C.
5) Wash the beads 4 times with Wash Buffer (+) (centrifuge the tube at 2,000 x g for 1 min.).
6) Add 400 µL of Master mix solution (Solution I: Solution II = 10 µL: 390 µL). Vortex thoroughly, then spin-down.
7) Add 250 µL of Solution III. Vortex thoroughly. Centrifuge the tube at 2,000 x g for 2 min.
8) Transfer the supernatant to the tube containing 2 µL of Solution IV.
9) Add 600 µL of ice-cold 2-propanol and place at -20°C for 20 min. Centrifuge the tube at 12,000 x g for 10 min.
10) Wash the pellet 2 times with 0.5 mL of ice-cold 70% ethanol and let the pellet dry for 5-15 min.
11) Dissolve the pellet in nuclease-free water.
12) Quantify the isolated RNA using NanoDrop (Thermo Fisher Scientific Inc.) and check the quality of RNA with Bioanalyzer (Agilent Technologies, Inc.).

(Positive control for RNP immunoprecipitation; HeLa)





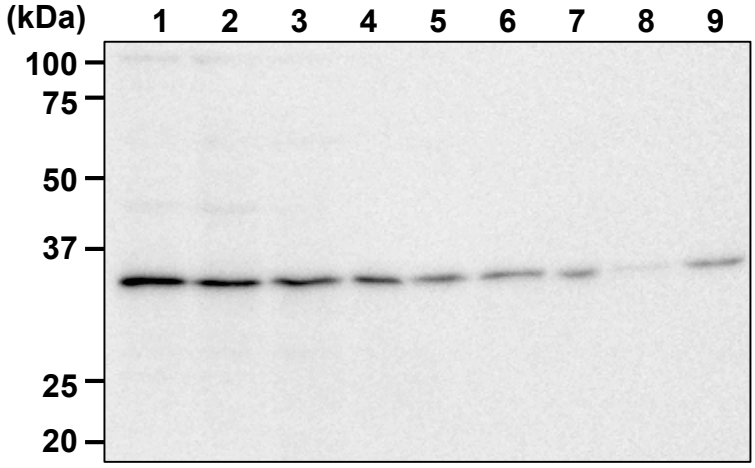
Analysis of RNA with Bioanalyzer

Average of the RNA Quantity (n=2)	
Antibody	RNA (ng)
Mouse IgG2a (M076-3)	47.8
Anti-ELAVL1(HuR) mAb (RN007M)	285.7
Total RNA	259805.0

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, then sonicate briefly (up to 20 sec.).
2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
3) Boil the samples for 3 min. and centrifuge. Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
4) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer's manual for precise transfer procedure.
5) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
6) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) [5 min. x 3 times].
7) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
8) Wash the membrane with PBS-T (10 min. x 3 times).
9) Incubate the membrane with the 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 hr. at room temperature.
10) Wash the membrane with PBS-T (10 min. x 3 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
12) Expose for 5 min. with ImageQuant LAS 4000 imaging system (Fujifilm). The condition for exposure may vary.

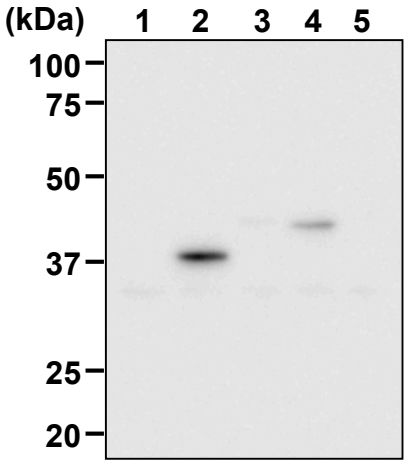
(Positive controls for Western blotting; 293T, HeLa, K562, Jurkat, NIH/3T3, WR19L, Rat1, CHO and transfectant)



Western blot analysis of ELAVL1

Lane 1: 293T
Lane 2: HeLa
Lane 3: K562
Lane 4: Jurkat
Lane 5: NIH/3T3
Lane 6: WR19L
Lane 7: Rat1
Lane 8: PC12
Lane 9: CHO

Immunoblotted with Anti-ELAVL1 (HuR) mAb (RN007M)



Western blot analysis of ELAV family proteins

Lane 1: 293T (Native cells)
Lane 2: ELAVL1 (HuR)/293T
Lane 3: ELAVL2 (HuB)/293T
Lane 4: ELAVL3 (HuC)/293T
Lane 5: ELAVL4 (HuD)/293T

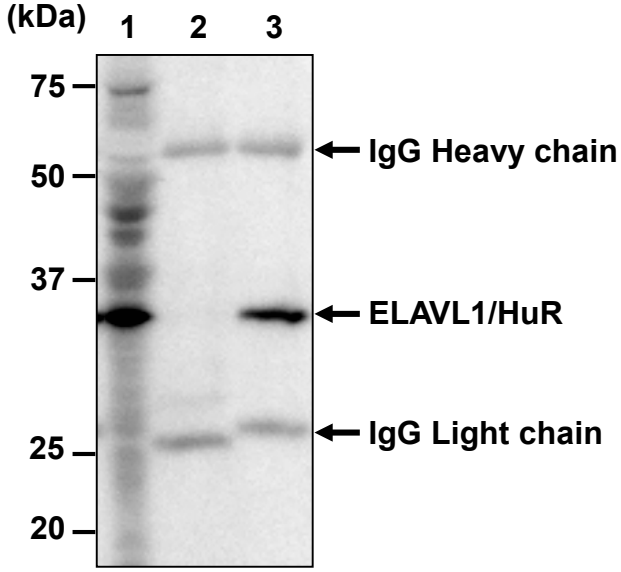
Loading volume: 1 x 10^4 cells/lane
Exposure time: 3 min.

Immunoblotted with Anti-ELAVL1 (HuR) mAb (RN007M)

Immunoprecipitation

1) Wash 1.65 x 10^7 cells 4 times with PBS and resuspend them with 500 µL of ice-cold Lysis Buffer (+) (MBL; code no. RN1001) containing appropriate protease inhibitors and DTT. Vortex thoroughly, then incubate on ice for 10 min.
2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
3) Add 25 µL of 50% protein G agarose beads slurry resuspended in Lysis Buffer (+) into the supernatant. Incubate at 4°C with rotating for 1 hr.
4) Centrifuge the tube at 2,000 x g for 1 min. at 4°C and transfer the pre-cleared supernatant to another tube.
5) Mix 25 µL of 50% protein G agarose beads slurry resuspended in 1 mL of ice-cold Lysis buffer (+) with primary antibody as suggested in the **APPLICATIONS**. Incubate at 4°C with rotating for 1 hr.
6) Wash the beads 1 time with ice-cold Lysis Buffer (+). Carefully discard the supernatant.
7) Add 50 µL of the pre-cleared supernatant to the tube containing antibody conjugated beads, then incubate with gentle agitation for 3 hr. at 4°C.
8) Wash the beads 4 times with 1 mL of ice-cold Wash Buffer (+) (MBL; code no. RN1001) containing DTT at the appropriate concentration.
9) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3 min. and centrifuge.
10) Load 20 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
11) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer's manual for precise transfer procedure.
12) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
13) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) [5 min. x 3 times].
14) Incubate the membrane with 1 µg/ml of anti-ELAVL1 (HuR) mAb (MBL; code no. RN007M) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
15) Wash the membrane with PBS-T (10 min. x 3 times).
16) Incubate the membrane with 1:1000 of Mouse TrueBlot® ULTRA: Anti-Mouse Ig HRP (eBioscience; code no. 18-8817-33) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
17) Wash the membrane with PBS-T (5 min. x 4 times).
18) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min.
19) Expose for 3 min. with ImageQuant LAS 4000 imaging system (Fujifilm). The condition for exposure may vary.

(Positive control for Immunoprecipitation; HeLa)



Immunoprecipitation of ELAVL1 from HeLa cells

Lane 1: Input (2% of total cell lysate)
Lane 2: Mouse IgG2a (M076-3)
Lane 3: Anti-ELAVL1 (HuR) mAb (RN007M)

Immunoblotted with RN007M