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Not for use in diagnostic procedures.

MBL
A JSR Life Sciences Company

RiboCluster Profiler™ **RIP-Certified Antibody**

Anti-FMR1 pAb

Code No.	Quantity	Concentration	Form
RN016P	200 µL	1 mg/mL	Affinity Purified

BACKGROUND: The fragile X mental retardation 1 protein (FMR1) is a cytoplasmic RNA binding protein abundantly expressed in the brain neurons. The absence of FMR1 protein leads to fragile X syndrome associated with the expansion of trinucleotide 5′-d(CGG)-3′ repeats within the FMR1 gene. Three RNA binding domains including 1 RGG box and 2 KH domains were found in FMR1. Besides the presence of RNA binding motifs, FMR1 was also found to carry nuclear localization and nuclear export signals and it is believed to shuttle in and out the nucleus. FMR1 associates with polyribosomes through RNA and it is suggested to act as both a negative regulator and an activator of mRNA translation. Furthermore, recent studies showed that FMR1 can associate with miRNA and the components of the miRNA pathway *in vivo*.

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, corresponding to C-terminus of human FMR1.

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 -20ºC.

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

REACTIVITY: This antibody reacts with human FMR1 on Western blotting, Immunoprecipitation and RNP Immunoprecipitation.

APPLICATIONS:
RNP Immunoprecipitation; 15 µg/500 µL of cell extract from 7 x 10^6 cells
Western blotting; 1 µg/mL
Immunoprecipitation; 5 µg/500 µL of cell extract from 5 x 10^6 cells
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

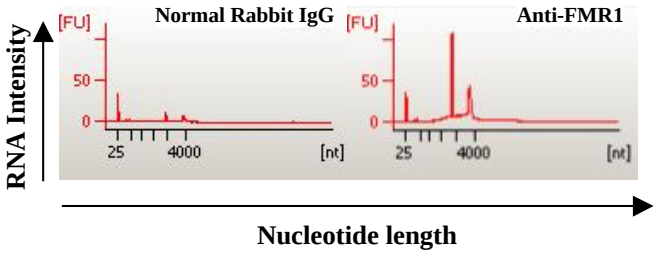
Detailed procedures are provided in the following **PROTOCOLS**.

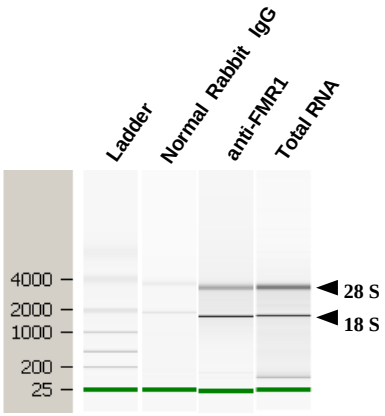
REFERENCES:
1) Fähling, M., *et al., J. Biol. Chem*. **284**, 4255-4266 (2009)
2) Bassell, G. J., and Warren, S. T., *Neuron* **60**, 201-214 (2008)
3) Jin, P., *et al., Nat. Neurosci*. **7**, 113-117 (2004)

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster
Cells	293T, HeLa, K562, Jurkat	NIH/3T3, WR19L	Rat1	Not Tested
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	47.5
anti-FMR1	193.0
Total RNA	123295.0

PROTOCOLS:

RNP Immunoprecipitation

Some buffers 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.

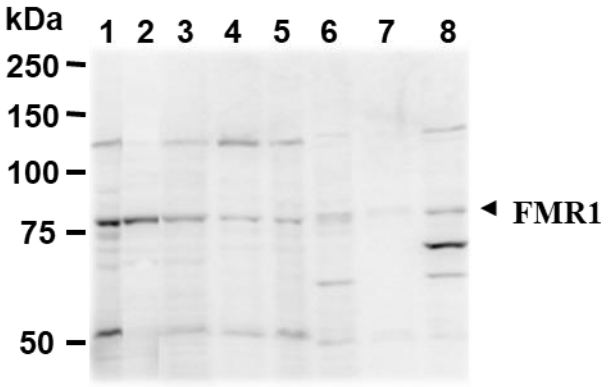
[Material Preparation]

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.

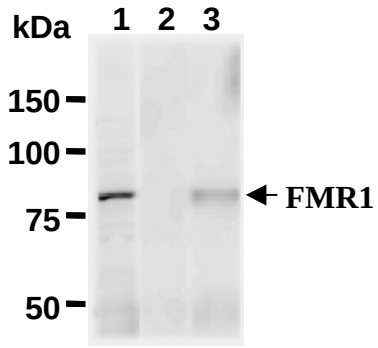
Protocol

1) Wash 7 x 10^6 cells 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 it on ice for 10 minutes.
2) Centrifuge the tube at 12,000 x g for 5 minutes at 4ºC 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 4ºC with rotating for 1 hour.
4) Centrifuge the tube at 2,000 x g for 1 minute at 4ºC and transfer the supernatant to another fresh tube (precleared sample).
5) Mix 25 µL of 50% protein A agarose beads slurry resuspended in nuclease-free PBS with Normal Rabbit IgG (RIP-Assay Kit) or anti-FMR1 antibody at the concentration suggested in the **APPLICATIONS,** and then add 1 mL of Wash buffer (+) into each tube. Incubate with gently agitation for 1 hour at 4ºC.
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 4ºC.
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 µL of Master mix solution (Solution I: Solution II = 10 µL: 390 µL). Vortex thoroughly, then spin-down.
15) Add 250 µL of 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 Solution IV.
18) Add 600 µL of ice-cold 2-propanol and place at -20ºC 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; HeLa)



Western blotting analysis of FMR1 expression in 293T (1), HeLa cytoplasm (2), HeLa whole lysate (3), K562 (4), Jurkat (5), NIH/3T3 (6), WR19L (7) and Rat1 (8) using RN016P.



Immunoprecipitation of FMR1 from HeLa with normal rabbit IgG (2) or RN016P (3).
After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with RN016P. Lane 1 is the input sample.

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 4ºC.
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 times).
7) Incubate the membrane with the 1:10,000 HRP-conjugated anti-rabbit IgG (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 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, K562, Jurkat, NIH3T3, WR19L, Rat1)

Immunoprecipitation

1) Wash cells (approximately 1 x 10^7 cells) 2 times with PBS and resuspend them with 1 mL of ice-cold Lysis buffer (RIP-Assay Kit) 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 4ºC 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 4ºC with rotating for 1 hour.
4) Centrifuge the tube at 2,000 x g for 1 minute at 4ºC and transfer the supernatant to another tube (precleared sample).
5) Mix 20 µL of 50% protein A agarose beads slurry resuspended in PBS with Normal Rabbit IgG (RIP-Assay Kit) or anti-FMR1 antibody at the concentration suggested in the **APPLICATIONS,** and then add 1 mL of Wash buffer into each tube. Incubate with gently agitation for 1 hour at 4ºC.
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 4ºC.
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 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)

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