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

MBL

RiboCluster Profiler™

Anti-PABPN1

Code No.	Quantity	Concentration	Form
RN023PW	100 µL	1 mg/mL	Affinity Purified

BACKGROUND: In mammals, the proteins of the poly (A)-binding protein (PABP) family exist in several cytoplasmic forms and one nuclear isoform, and these proteins are encoded by different genes. PABP binds to the mRNA poly(A) tail in the cytoplasm and regulates both mRNA stability and translation, while PABPN1 in the nucleus is involved in the synthesis of the poly(A) tail, regulation of the length of newly synthesized poly(A) tail, and stimulating the maturation of mRNA. Immunofluorescence microcopy revealed that PABPN1 is localized throughout the nucleoplasm, with higher concentration in speckles, and that the binding of PABPN1 to poly(A) tails is essential for its localization to the speckles. It has been reported that PABPN1 binds to the RNA polymerase II before, at the start of, or shortly after the initiation of transcription; therefore, the assembly of PABPN1 onto the poly(A) tail may be coupled with transcription. Abnormal expansion of a $(GCG)_6$ trinucleotide repeat at the 5′ end of the coding region of *PABPN1* gene may lead to autosomal dominant oculopharyngeal muscular dystrophy (OPMD). Intranuclear inclusions of PABPN1 may play important roles in the pathogenesis of this disease, since the deletion of the C-terminal oligomerization domain in PABPN1 inactivates its oligomerization and reduces cell death.

SOURCE: This antibody was purified from rabbit serum by affinity column chromatography. The rabbit was immunized with KLH conjugated synthetic peptide, corresponding to internal region of human PABPN1.

FORMULATION: 100 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 and mouse PABPN1 on Western blotting and Immunoprecipitation.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; 5 µg/500 µL of cell extract from 5 x 10^6 cells
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested
RNP Immunoprecipitation; Not tested

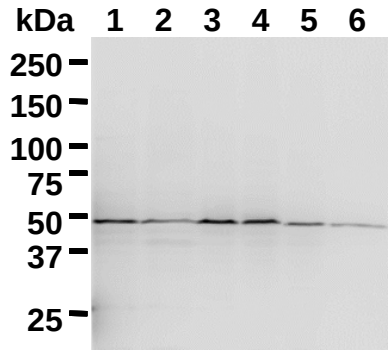
Detailed procedure is provided in the following **PROTOCOLS**.

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

REFERENCES:
1) Hino, H., *et al*., *Hum. Mol. Genet*. **13,** 181-190 (2004)
2) Mangus, D. A., *et al*., *Genome Biol*. **4,** 223 (2003)
3) Bear, D. G., *et al., Exp. Cell Res*. **286,** 332-334 (2003)
4) Calado, A., and Carmo-Fonseca, M., *J. Cell Sci*. **113**, 2309-2318 (2000)

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster
Cells	293T, HeLa, K562, Jurkat	NIH/3T3, WR19L	Not Tested	Not Tested
Reactivity on WB	+	+		



Western blot analysis of PABPN1 expression in 293T (1), HeLa (2), K562 (3), Jurkat (4), NIH/3T3 (5) and WR19L (6) using RN023PW.

PROTOCOLS:

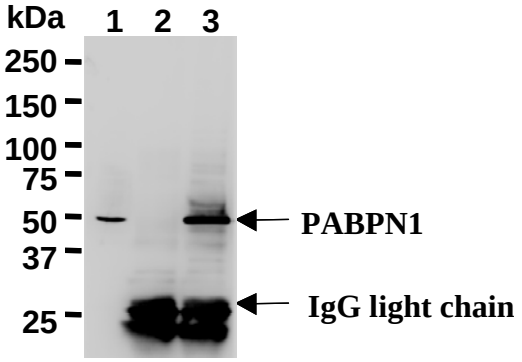
SDS-PAGE & Western Blotting

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% MeOH). 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 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 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, Jurkat, K562, NIH/3T3, WR19L)



Immunoprecipitation of PABPN1 from HeLa nucleus with normal rabbit IgG (2) or RN023PW (3). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with RN023PW. Lane 1 is the input sample.*

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 4°C and discard the supernatant.
3) Wash the pellet 3 times with PBS and resuspend them with 300 µL RIPA Buffer, then sonicate briefly.
4) Centrifuge the tube at 12,000 x g for 5 minutes at 4°C and transfer the supernatant to another fresh tube.
5) Add 700 µL ice-cold Lysis buffer to the nuclear extract prepared from step 4.
6) Mix 20 µL of 50% protein A agarose beads slurry resuspended in PBS with Normal Rabbit IgG (RIP-Assay Kit) or anti-PABPN1 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 4°C.
7) 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.
8) Add 500 µL of nuclear extract (the sample from step 5), then incubate with gentle agitation for 3 hours at 4°C
9) Wash the beads 4 times with Wash Buffer (centrifuge the tube at 2,000 x g for 1 minute).
10) 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 nucleus)

RELATED PRODUCTS:

RIP-Assay Kit

RN1001	RIP-Assay Kit

RIP Certified Antibody

RN001P	Anti-EIF4E (polyclonal)
RN002P	Anti-EIF4G1 (polyclonal)
RN003P	Anti-EIF4G2 (polyclonal)
RN004P	Anti-ELAVL1/HuR (polyclonal)
RN005P	Anti-ELAVL2/HuB (polyclonal)
RN006P	Anti-ELAVL3/HuC (polyclonal)
RN007P	Anti-IGF2BP1/IMP1 (polyclonal)
RN008P	Anti-IGF2BP2/IMP2 (polyclonal)
RN009P	Anti-IGF2BP3/IMP3 (polyclonal)
RN010P	Anti-MSI1/Musashi1 (polyclonal)

Other RIP-Certified Antibodies are also available.
Please visit our website at
https://ruo.mbl.co.jp/product/epigenetics/rip-assay.html

RIP-Assay Starter Kit

Each RIP-Assay Starter Kit contains 40 µg of RIP-Certified Antibody and RIP-Assay Kit.

RN001PK	RIP-Assay Starter Kit EIF4E (polyclonal)
RN002PK	RIP-Assay Starter Kit EIF4G1 (polyclonal)
RN003PK	RIP-Assay Starter Kit EIF4G2 (polyclonal)
RN004PK	RIP-Assay Starter Kit ELAVL1/HuR (polyclonal)
RN005PK	RIP-Assay Starter Kit ELAVL2/HuB (polyclonal)
RN006PK	RIP-Assay Starter Kit ELAVL3/HuC (polyclonal)
RN007PK	RIP-Assay Starter Kit IGF2BP1/IMP1 (polyclonal)
RN008PK	RIP-Assay Starter Kit IGF2BP2/IMP2 (polyclonal)
RN009PK	RIP-Assay Starter Kit IGF2BP3/IMP3 (polyclonal)
RN010PK	RIP-Assay Starter Kit MSI1/Musashi1 (polyclonal)

Other RIP-Assay Starter Kits are also available.
Please visit our website at
https://ruo.mbl.co.jp/product/epigenetics/rip-assay.html

RBP Antibody

RBP Antibody works on WB and /or IP, but not certified for working on RIP-Assay.

RN023PW	Anti-PABPN1 (polyclonal)
RN028PW	Anti-EIF2C1/AGO1 (polyclonal)
RN029PW	Anti-EIF2C2/AGO2 (polyclonal)
RN030PW	Anti-DICER1 (polyclonal)
RN031PW	Anti-ZFP36 (polyclonal)
RN034PW	Anti-CUGBP1 (polyclonal)
RN035PW	Anti-CUGBP2 (polyclonal)