

**RiboCluster Profiler™**

RIP-Certified Antibody

## Anti-YBX1 pAb

Code No.  
RN015P

Quantity  
200 µL

Form  
Affinity Purified

**BACKGROUND:** The Y-box-binding protein (YBX1), family of cold shock proteins, is multifunctional and appears to regulate gene expression at both the transcriptional and posttranscriptional levels. Transcriptional targets for YBX1 includes genes associated with cell death, cell death associated receptor and tumor suppressor genes. YBX1 also up regulates genes associated with cell proliferation. YBX1 is also implicated to have an important role in inducing drug resistance on tumor cells. YBX1 binds RNAs sequence specific manner and interacts with splicing factors and plays important role in mRNA splicing and translation. Recent study suggested that competition between YBX1 and eIF4G for mRNA binding is required for efficient stimulation of eIF4F activity by PABP.

### 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, QNYQNSESGEKNEGSES corresponding to 160-176 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.

### INTENDED USE:

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

**REACTIVITY:** This antibody reacts with human YBX1 (~36 kDa) on Western blotting, Immunoprecipitation and RNP Immunoprecipitation.

### APPLICATIONS:

RNP Immunoprecipitation; 15 µL/500 µL of cell extract from  $1.5 \times 10^7$  cells

Western blotting; 1:1,000

Immunoprecipitation; 5 µL/250 µL of cell extract from  $2.5 \times 10^6$  cells

Immunohistochemistry; Not tested

Immunocytochemistry; Not tested

Flow cytometry; Not tested

Chromatin Immunoprecipitation; Not tested\*

\*It is reported that this antibody is used in this application in the reference number 2).

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

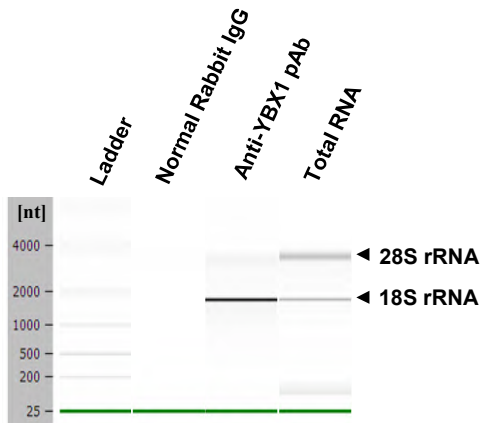
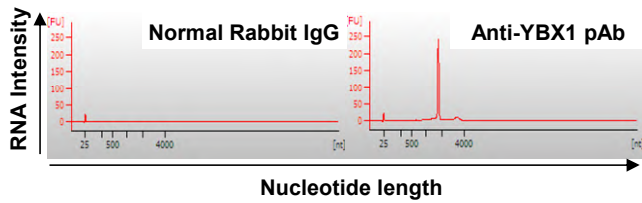
### REFERENCES:

- 1) Somasekharan, S. P., *et al.*, *J. Cell Biol.* **208**, 913-929 (2015) [RIP]
- 2) Kotake, Y., *et al.*, *Genes Cell* **18**, 999-1006 (2013) [ChIP]
- 3) Svitkin, Y. V., *et al.*, *EMBO J.* **28**, 58-68 (2009)
- 4) Vaiman, A. V., *et al.*, *Biochemistry(Mosc)* **71**, 146-154 (2006)
- 5) Homer, C., *et al.*, *Oncogene* **24**, 8314-8325 (2005)

### SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster
Cells	K562, HEK293T, 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=2)	
Antibody	RNA (ng)
Normal Rabbit IgG	53.2
Anti-YBX1 pAb	2079.5
Total RNA	235215.0

## 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]

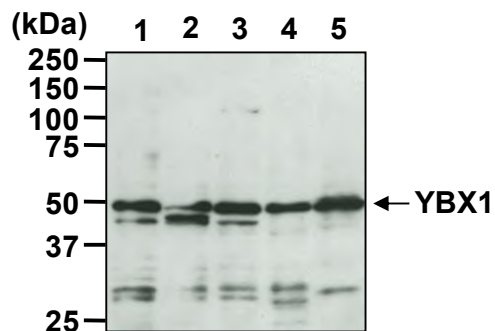
1. Lysis Buffer (+)  
Before using the Lysis Buffer, protease inhibitors, RNase inhibitors, and DTT are added to the Lysis Buffer at the appropriate concentration.
2. Wash Buffer (+)  
Before using the Wash Buffer, DTT is added to the Wash Buffer at the appropriate concentration.

#### Protocol

- 1) Wash  $1.5 \times 10^7$  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-YBX1 pAb (RN015P) at the amount of 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.
- 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  $\mu$ L 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  $\mu$ L of Master mix solution (Solution I: Solution II = 10  $\mu$ L: 390  $\mu$ L). Vortex for 10 seconds.
- 10) Add 250  $\mu$ L 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  $\mu$ L of Solution IV.
- 13) 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.
- 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; HEK293T)



**Western blot analysis of YBX1 expression in HEK293T (1), HeLa (2), NIH/3T3 (3), Rat1 (4) and CHO (5) using RN015P.**

### 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% MeOH). See the manufacture's manual for precise transfer procedure.

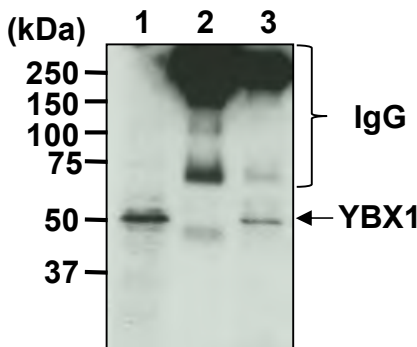
- 4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
  - 5) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggested in the **APPLICATIONS** for 1 hour at room 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.
- 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-YBX1 pAb (RN015P) at the amount of 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.
  - 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 (non-reducing condition), boil for 3-5 minutes, and centrifuge for 5 minutes. Use 20 µL/lane for the SDS-PAGE analysis. (See SDS-PAGE & Western blotting.) For 2nd antibody reaction, use 1:1,000 of True blot®: Anti-Rabbit IgG HRP (Rockland Immunochemicals; code no. 18-8816-31).

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

(Positive control for Immunoprecipitation; HeLa)

#### RELATED PRODUCTS:

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



**Immunoprecipitation of YBX1 from HeLa with normal rabbit IgG (2) or RN015P (3).** After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with RN015P. Input sample was loaded in lane 1.

#### 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 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).