

# Anti-Inosine pAb

<b>CODE No.</b>	PM098
<b>CLONALITY</b>	Polyclonal
<b>ISOTYPE</b>	Rabbit Ig, affinity purified
<b>QUANTITY</b>	100 µL
<b>SOURCE</b>	Purified Ig from rabbit serum
<b>FORMULATION</b>	PBS containing 50% Glycerol (pH 7.2). No preservative is contained.
<b>STORAGE</b>	This antibody solution is stable for one year from the date of purchase when stored at -20°C.

## APPLICATIONS-CONFIRMED

<u>RNA immunoprecipitation</u>	10 µL/sample
<u>Immunocytochemistry</u>	1:100-1:200
<u>Dot blotting</u>	Can be used.
<u>RNA ELISA</u>	Can be used.

## APPLICATION-UNDER EVALUATION

<u>Immunohistochemistry</u>	Can be used.
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## REFERENCES

- 1) Nishikura, K., *Nat. Rev. Mol. Cell Biol.* **17**, 83-96 (2016)
- 2) Nigita, G., *et al.*, *Front. Bioeng. Biotechnol.* **3**, 37 (2015)
- 3) Zinshteyn, B. and Nishikura, K., *Wiley Interdiscip. Rev. Syst. Biol. Med.* **1**, 202-209 (2009)
- 4) Morse, D. P. and Bass, B. L., *Biochemistry* **36**, 8429-8434 (1997)

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## RELATED PRODUCTS

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

## **RNA immunoprecipitation**

Some buffers and reagents are included in the RIP-Assay Kit for *microRNA* (code. RN1005). Please also refer to the protocol packaged in the RIP-Assay Kit for *microRNA*.

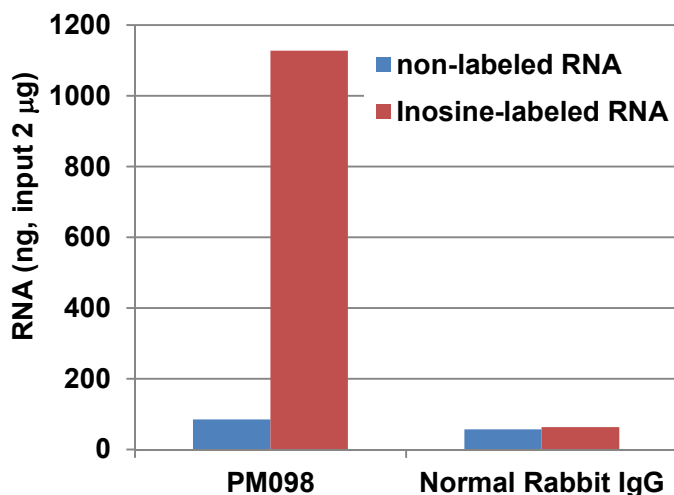
### [Material Preparation]

1. ***RNA-IP Buffer (+)*** [mi-Lysis Buffer (component of RN1005) containing 1.5 mM DTT and RNase inhibitor]  
Before using RNA-IP Buffer (+), RNase inhibitor and DTT are added to mi-Lysis Buffer at the appropriate concentration.
2. ***Wash Buffer*** [mi-Wash Buffer (component of RN1005) containing 1.5 mM DTT]  
Before using Wash Buffer, DTT is added to mi-Wash Buffer at the appropriate concentration.
3. Antibody conjugated Protein G beads
  - A) Mix 20  $\mu$ L of 50% protein G agarose beads slurry resuspended in nuclease-free PBS with 600  $\mu$ L of mi-Wash Buffer (component of RN1005), and then add Normal Rabbit IgG (component of RN1005) or Anti-Inosine pAb (PM098) at the concentration suggested in the **APPLICATIONS**. Incubate with gentle agitation overnight at 4°C.
  - B) Wash the beads 1 time with mi-Lysis Buffer (component of RN1005) containing 1.5 mM DTT.
  - C) Carefully discard the supernatant using a pipettor without disturbing the beads and incubate at 4°C until just before use.
4. Input total RNA  
Prepare total RNA samples by appropriate isolation method. Heat-denature the total RNA samples at 80°C for 2 min., then quench at 4°C for more than 5 min.

### [Protocol (RNA isolation; 2-step method in RN1005)]

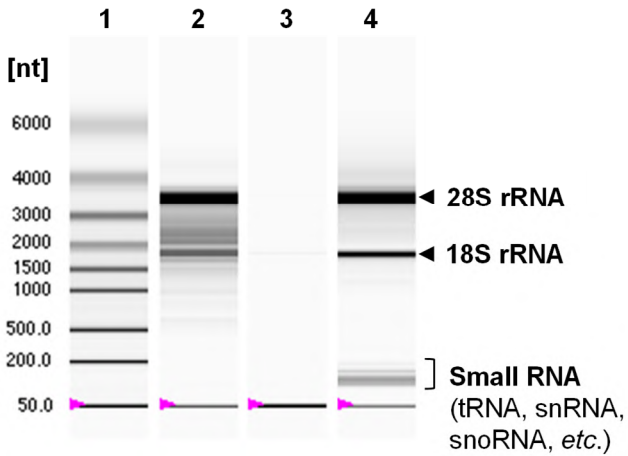
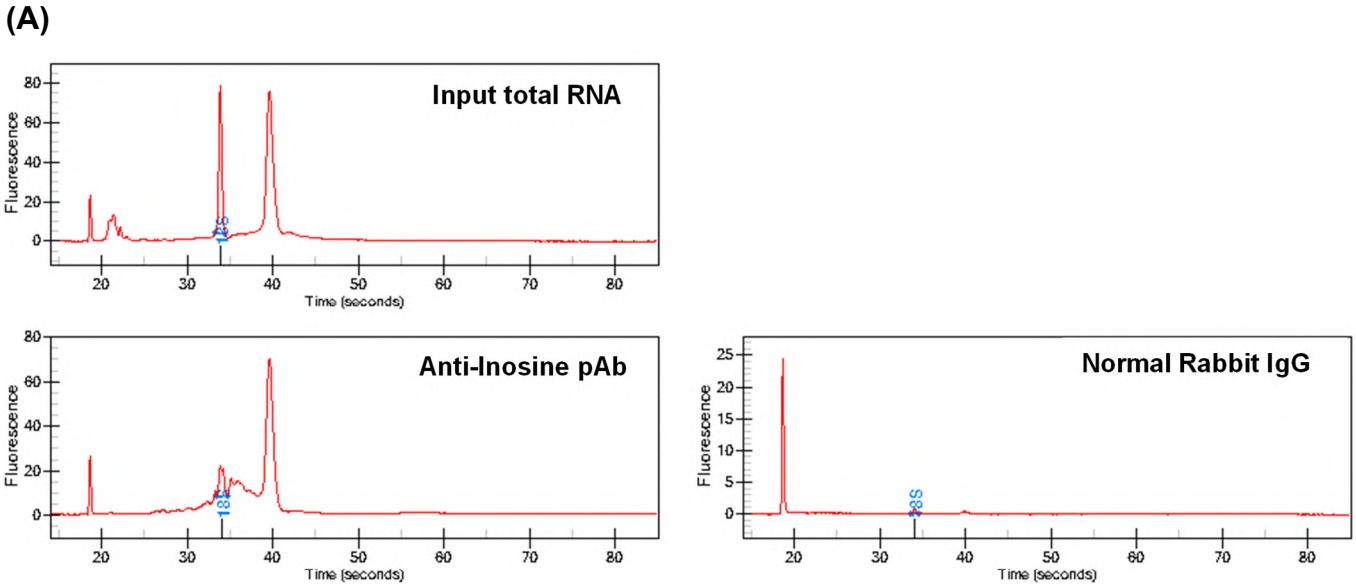
- 1) Add 40  $\mu$ g of input total RNA and 500  $\mu$ L of RNA-IP Buffer (+) into the tube containing antibody conjugated beads, then incubate with gentle agitation for 3 hr. at 4°C.
- 2) Wash the beads 4 times with 1 mL of Wash Buffer (centrifuge the tube at 2,000 x g for 1 min.).
- 3) Add 250  $\mu$ L of Master mix solution (mi-Solution I: mi-Solution II = 10  $\mu$ L: 240  $\mu$ L). Vortex thoroughly, then spin-down.
- 4) Add 150  $\mu$ L of mi-Solution III. Vortex thoroughly.
- 5) Centrifuge the tube at 2,000 x g for 2 min.
- 6) Transfer the supernatant to the new tube containing 2  $\mu$ L of mi-Solution IV.
- 7) Add 400  $\mu$ L of ice-cold 100% ethanol. Vortex thoroughly, then spin-down. Place at -20°C for 20 min. Centrifuge the tube at 12,000 x g for 10 min. at 4°C, then add 2  $\mu$ L of mi-Solution IV to the supernatant in the same tube.
- 8) Add 400  $\mu$ L of ice-cold 100% ethanol. Vortex thoroughly, then spin-down. Place at -20°C for 20 min. Centrifuge the tube at 12,000 x g for 10 min. at 4°C.
- 9) Wash the pellet 2 times with 500  $\mu$ L of ice-cold 70% ethanol and dry up the pellet for 5-15 min.
- 10) Dissolve the pellets in 20  $\mu$ L of nuclease-free water. Quantify the isolated RNA using NanoDrop (Thermo Fisher Scientific Inc.) and check the quality of RNA with Experion (Bio-Rad).

(Positive control for RNA immunoprecipitation; HEK293T total RNA)



### ***RNA immunoprecipitation from in vitro transcribed RNA***

Sample: RNA synthesized by *in vitro* transcription from *lacZ*-encoding cDNA (RefSeq ID: NC\_007779.1, region 363130-364149)



Lane 1: Ladder  
 Lane 2: Anti-Inosine pAb  
 Lane 3: Normal Rabbit IgG  
 Lane 4: Input total RNA

(B)

Average of the RNA Quantity (n=2)	
Antibody	RNA (ng)
Anti-Inosine pAb	594.4
Normal Rabbit IgG	70.8

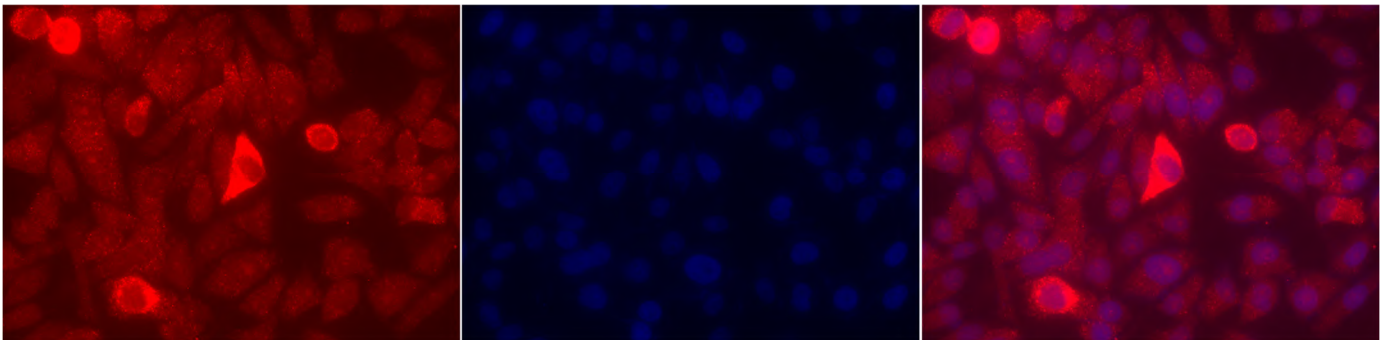
**RNA immunoprecipitation from HEK293T total RNA**

- (A) Characterization of isolated RNA with Experion
- (B) Quantification of isolated RNA with NanoDrop

### **Immunocytochemistry**

- 1) Spread cells on a glass chamber slide, then incubate in a CO<sub>2</sub> incubator for one night.
- 2) Remove the culture supernatant by careful aspiration.
- 3) Wash the slide with PBS.
- 4) Fix the cells with 4% paraformaldehyde in PBS for 10 min. at room temperature (20~25°C).
- 5) Wash the slide 3 times with PBS.
- 6) Permeabilize the cells with 0.5% Triton X-100 in PBS for 10 min. at room temperature.
- 7) Wash the slide 3 times with PBS.
- 8) Block the cells with 5% BSA in PBS-T [0.05% Tween-20 in PBS] for 1 hr. at room temperature.
- 9) Incubate the cells with the primary antibody diluted with PBS as suggested in the **APPLICATIONS** for 2 hr. at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
- 10) Wash the slide 3 times with PBS-T.
- 11) Incubate the cells with 1:200 of Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed, Alexa Fluor® 594 (Thermo Fisher Scientific; code no. A11037) for 1 hr. at room temperature.
- 12) Wash the slide 3 times with PBS-T.
- 13) Mount the slide with Mount medium with DAPI.

(Positive control for Immunocytochemistry; HeLa)



### ***Immunocytochemistry in HeLa cells***

Red: Anti-Inosine pAb (PM098), 1:200  
Blue: DAPI