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Anti-Inosine pAb

CODE No.	PM098
CLONALITY	Polyclonal
ISOTYPE	Rabbit Ig, affinity purified
QUANTITY	100 µL
SOURCE	Purified Ig from rabbit serum
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-CONFIRMED

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

APPLICATION-UNDER EVALUATION

Immunohistochemistry	Can be used.

REFERENCES

1) Nishikura, K., *Nat. Rev. Mol. Cell Biol.* **17**, 83-96 (2016)
2) Nigita, G., *et al., Front. Bioeng. Biotechonol*. **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*.

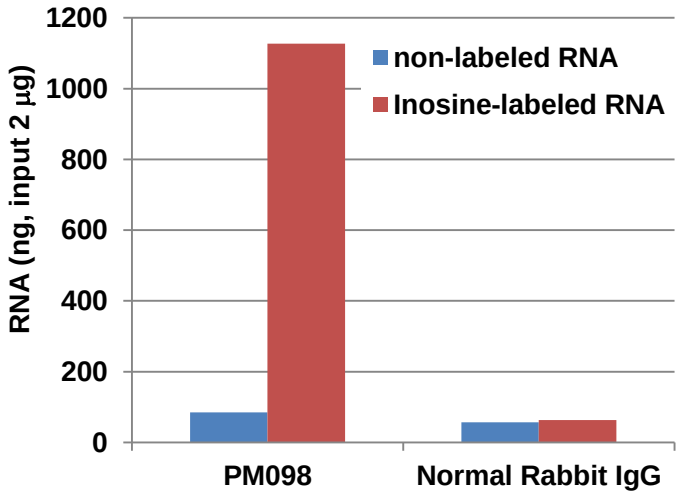
[Material Preparation]

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 µL of 50% protein G agarose beads slurry resuspended in nuclease-free PBS with 600 µL 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 µg of input total RNA and 500 µL 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 µL of Master mix solution (mi-Solution I: mi-Solution II = 10 µL: 240 µL). Vortex thoroughly, then spin-down.
4) Add 150 µL 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 µL of mi-Solution IV.
7) Add 400 µL 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 µL of mi-Solution IV to the supernatant in the same tube.
8) Add 400 µL 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 µL of ice-cold 70% ethanol and dry up the pellet for 5-15 min.
10) Dissolve the pellets in 20 µL of nuclease-free water. Quantify the isolated RNA using NanoDrop (Thermo Fisher Scientific Inc.) and check the quality of RNA with Experion (Bio-Rad).

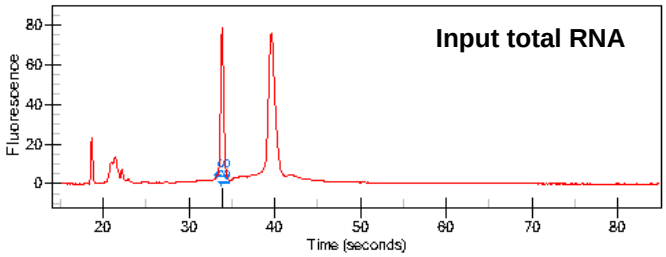
(Positive control for RNA immunoprecipitation; HEK293T total RNA)

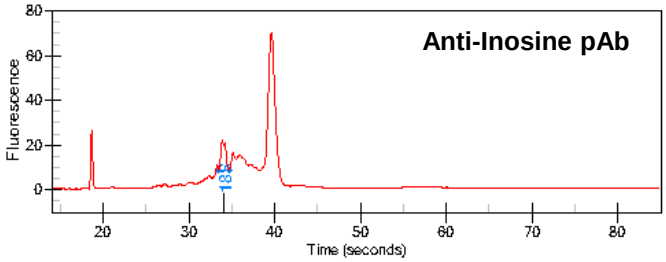


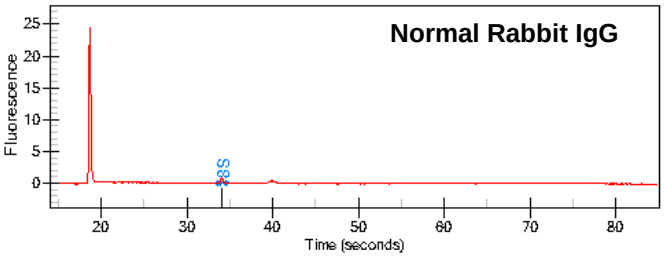
RNA immunoprecipitation from in vitro transcribed RNA

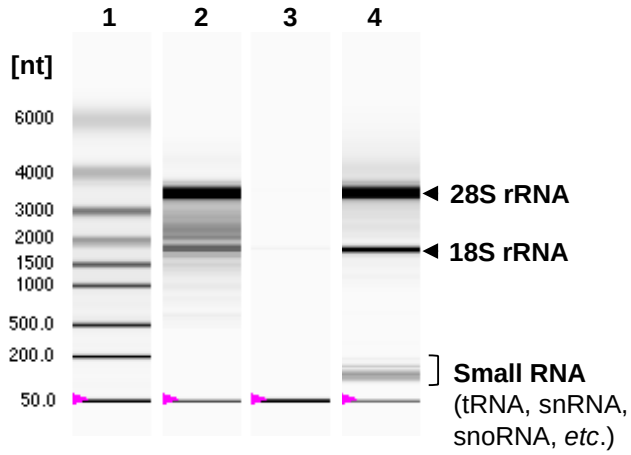
Sample: RNA synthesized by *in vitro* transcription from *lacZ*-encoding cDNA (RefSeq ID: NC_007779.1, region 363130-364149)

(A)









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

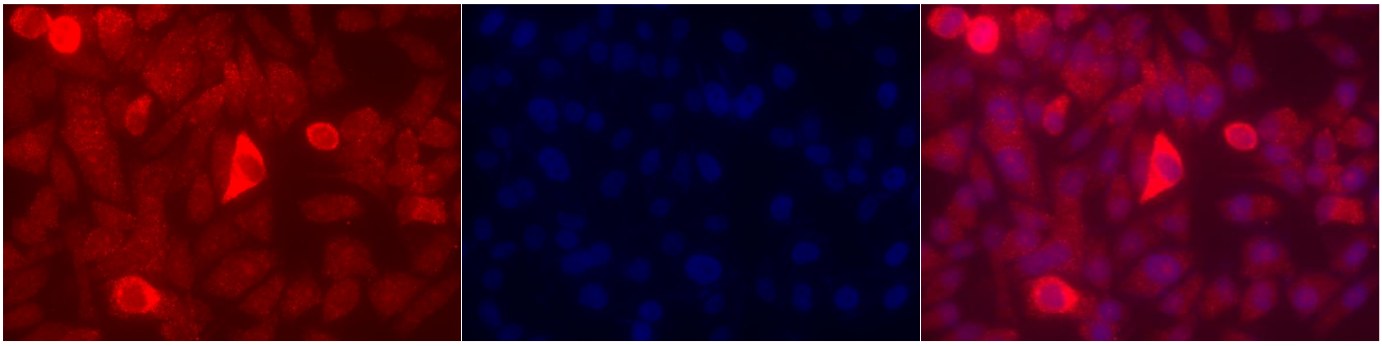
RNA immunoprecipitation from HEK293T total RNA

(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_2 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)



Immunocytochemstry in HeLa cells
Red: Anti-Inosine pAb (PM098), 1:200
Blue: DAPI