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**

For more information, please visit our web site [https://ruo.mbl.co.jp/](https://ruo.mbl.co.jp/).

**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 HEK293T total RNA

(A) Characterization of isolated RNA with Experion

(B) Quantification of isolated RNA with NanoDrop

<table>
<thead>
<tr>
<th>Antibody</th>
<th>RNA (ng)</th>
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<tbody>
<tr>
<td>Anti-Inosine pAb</td>
<td>594.4</td>
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<tr>
<td>Normal Rabbit IgG</td>
<td>70.8</td>
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Average of the RNA Quantity (n=2)
Immunocytochemistry

1) Spread cells on a glass chamber slide, then incubate in a CO₂ 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