

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



Anti-1-methyladenosine (m¹A) mAb

CODE No.	D345-3
CLONALITY	Monoclonal
CLONE	AMA-2
ISOTYPE	Mouse IgG2b κ
QUANTITY	100 μL, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	KLH-conjugated 1-methyladenosine (KLH-m ¹ A)
REACTIVITY	This clone reacts with 1-methyladenosine (m ¹ A or m ¹ Ado), 1-methyladenine (1-mA) and m ¹ A containing RNA such as tRNA.
FORMURATION	PBS containing 50% glycerol. 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

<u>Immunohistochemistry</u>	0.1 μg/mL (paraffin section)
<u>Immunocytochemistry</u>	1 μg/mL
<u>RNA immunoprecipitation</u>	15 μg/sample

REFERENCES	1) Mishima, E., <i>et al.</i> , <i>J. Am. Soc. Nephrol.</i> (2014) In press.
	2) Masuda, M., <i>et al.</i> , <i>Cancer</i> 72 , 3571-3578 (1993)
	3) Itoh, K., <i>et al.</i> , <i>Tohoku J. Exp. Med.</i> 168 , 329-331 (1992)
	4) Itoh, K., <i>et al.</i> , <i>Jpn. J. Cancer. Res.</i> 79 , 1130-1138 (1988)

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URL <http://ruo.mbl.co.jp/>
e-mail support@mbi.co.jp, TEL 052-238-1904

RELATED PRODUCTS

Antibodies

- D345-3 Anti-1-methyladenosine (m¹A) mAb (AMA-2)
- D346-3 Anti-5-methylcytidine (m⁵C) mAb (FMC-9)
- PM077 Anti-5-hydroxymethylcytosine (5hmC) pAb
- MI-11-3 Anti-Bromodeoxyuridine mAb (2B1)
- M077-3 Mouse IgG2b (isotype control)

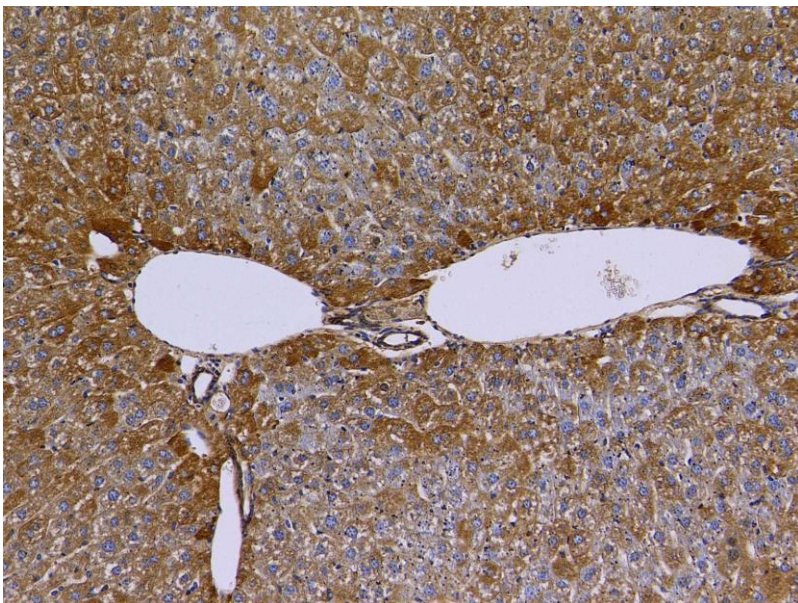
Kit

- RN1005 RIP-Assay Kit *for microRNA*

Immunohistochemistry for formalin fixed paraffin-embedded section

- 1) Deparaffinize the section with Xylene (5 min. x 3 times).
- 2) Wash the slide with Ethanol (5 min. x 3 times).
- 3) Wash the slide with PBS (5 min. x 3 times).
- 4) Remove the slide from PBS and inactivate endogenous peroxidase with 3% H₂O₂ in PBS for 10 min.
- 5) Wash the slide with PBS (5 min. x 3 times).
- 6) Remove the slide from PBS, wipe gently around each section and cover tissues with blocking buffer [20 mM HEPES/1% BSA/135 mM NaCl (pH 7.4)] for 5 min. at room temperature to block non-specific staining. Do not wash.
- 7) Tip off the blocking buffer, wipe gently around the section and incubate with primary antibody diluted with blocking buffer as suggest in the **APPLICATIONS** (The concentration of antibody will depend on the conditions.) for 1 hr. at room temperature.
- 8) Wash the slide with PBS (5 min. x 3 times).
- 9) Wipe gently around the section and incubate with Histostar (Ms + Rb) (MBL; code no. 8460) for 30 min. at room temperature.
- 10) Wash the slide with PBS (5 min. x 3 times).
- 11) Visualize by reacting for 5 min. with Histostar™ DAB Substrate Solution (MBL; code no. 8469). *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
- 12) Wash the slide in water for 5 min.
- 13) Counterstain in hematoxylin for 1 min., wash the slide 3 times in water for 5 min. each, and then immerse the slide in PBS for 5 min.
- 14) Dehydrate by immersing in Ethanol 3 times for 5 min. each, followed by immersing in Xylene 3 times for 5 min. each. Now ready for mounting.

(Positive control for Immunohistochemistry; Ischemic model mouse liver)



Immunohistochemistry in ischemic model mouse liver

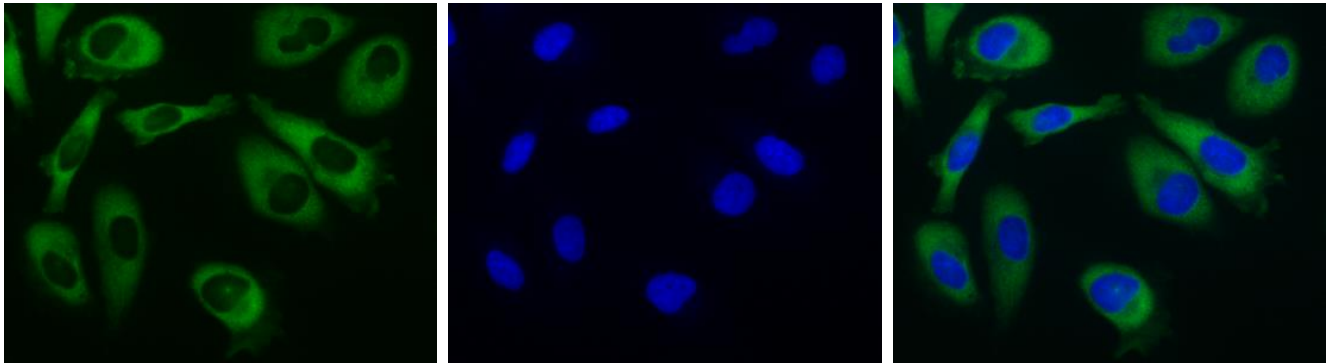
Brown: Anti-1-methyladenosine (m¹A) mAb (D345-3)
Blue: Hematoxylin

The sample was kindly provided by Dr. Takaaki Abe, *M.D., Ph.D.*
(Division of Nephrology, Endocrinology, and Vascular Medicine, Tohoku University Graduate School of Biomedical Engineering)

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 2 times with PBS.
- 4) Fix the cells with 4% paraformaldehyde (PFA)/PBS for 20 min. at room temperature (20~25°C).
- 5) Wash the slide 2 times with PBS.
- 6) Permeabilize the cells with 0.5% Triton X-100/PBS for 5 min. at room temperature.
- 7) Wash the slide 2 times with PBS.
- 8) Block the cells with blocking buffer (1% BSA/PBS) for 1 hr. at room temperature. *10% FBS/PBS is not recommended for blocking.
- 9) Tip off the blocking buffer and incubate the cells with the primary antibody diluted with blocking buffer as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
- 10) Wash the slide with 0.2% Tween20/PBS (5 min. x 3 times).
- 11) Incubate the cells with 1:1,000 Alexa Fluor[®] 488 Goat Anti-Mouse IgG (Invitrogen; code no. A11032) diluted with blocking buffer for 1 hr. at room temperature in dark chamber.
- 12) Wash the slide with 0.2% Tween20/PBS (5 min. x 3 times).
- 13) Counterstain with Hoechst33342 and observe the slide using fluorescent microscopy.

(Positive control for Immunocytochemistry; HeLa)



Immunocytochemistry in HeLa cells

Green: Anti-1-methyladenosine (m¹A) mAb (D345-3)
Blue: Hoechst33342

RNA immunoprecipitation

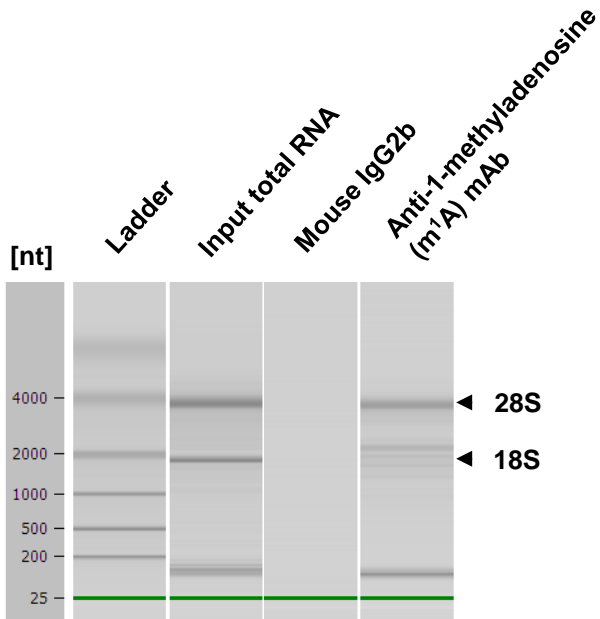
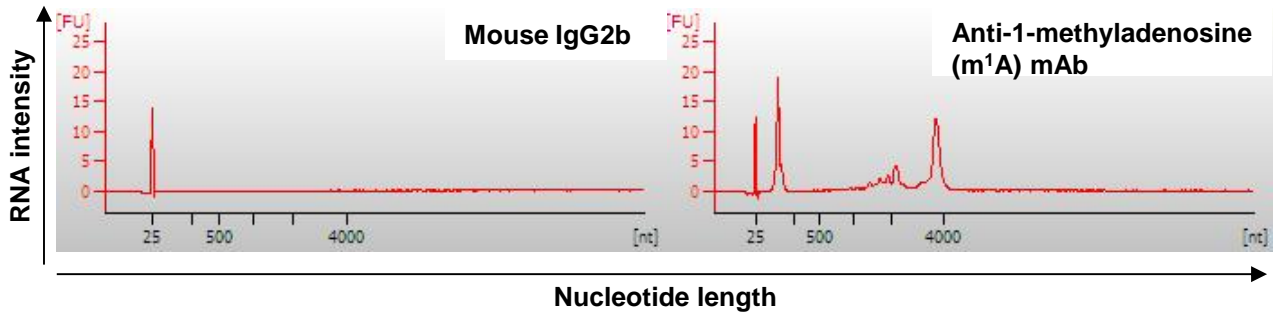
[Material Preparation]

1. ***RNA-IP Buffer*** [mi-Lysis Buffer (component of RN1005) containing 50 U/mL RNase inhibitor and 1.5 mM DTT]
Before using the 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 the Wash Buffer, DTT is added to mi-Wash Buffer at the appropriate concentration.
3. Antibody conjugated Protein G beads
 - A) Mix 20 μ L of 50% protein G agarose beads slurry resuspended in nuclease-free PBS with 600 μ L of Wash Buffer, and then add Mouse IgG2b (isotype control) (MBL; code no. M077-3) or Anti-1-methyladenosine (m¹A) mAb at the concentration suggested in the **APPLICATIONS**. Incubate with gentle agitation for 1 hr. at 4°C.
 - B) Wash the beads 1 time with ice-cold RNA-IP Buffer (centrifuge the tube at 2,000 x g for 1 min.). Carefully discard the supernatant using a pipettor without disturbing the beads and incubate at 4°C until just before use.
4. Total RNA
Prepare total RNA by appropriate isolation method. Heat the RNA sample 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 μ g of total RNA and 1 mL 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 μ L of Master mix solution (mi-Solution I: mi-Solution II = 10 μ L: 240 μ L). Vortex thoroughly, then spin-down.
- 4) Add 150 μ 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 μ L of mi-Solution IV.
- 7) Add 400 μ L of ice-cold 100% ethanol. Vortex thoroughly, then spin-down. Place at -20°C or below for 20 min. Centrifuge the tube at 12,000 x g for 10 min. at 4°C, then add 2 μ L of mi-Solution IV to the supernatant in the same tube.
- 8) Add 400 μ L of ice-cold 100% ethanol. Vortex thoroughly, then spin-down. Place at -20°C or below 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 μ L of ice-cold 70% ethanol and dry up the pellet for 5-15 min.
- 10) Dissolve the pellet in 20 μ L of nuclease-free water. Quantify the isolated RNA using NanoDrop (Thermo Fisher Scientific Inc.) and check the quality of RNA with Bioanalyzer (Agilent Technologies, Inc.).

(Positive control for RNA immunoprecipitation; HEK293T total RNA)



Analysis of RNA with Bioanalyzer

Average of the RNA Quantity (n=2)	
Antibody	RNA (ng)
Mouse IgG2b	40.0
Anti-1-methyladenosine (m ¹ A) mAb	320.0