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Not for use in diagnostic procedures.

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Anti-1-methyladenosine (m^1A) 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^1A)
REACTIVITY	This clone reacts with 1-methyladenosine (m^1A or m1Ado), 1-methyladenine (1-mA) and m^1A containing RNA such as tRNA.
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

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

REFERENCES

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

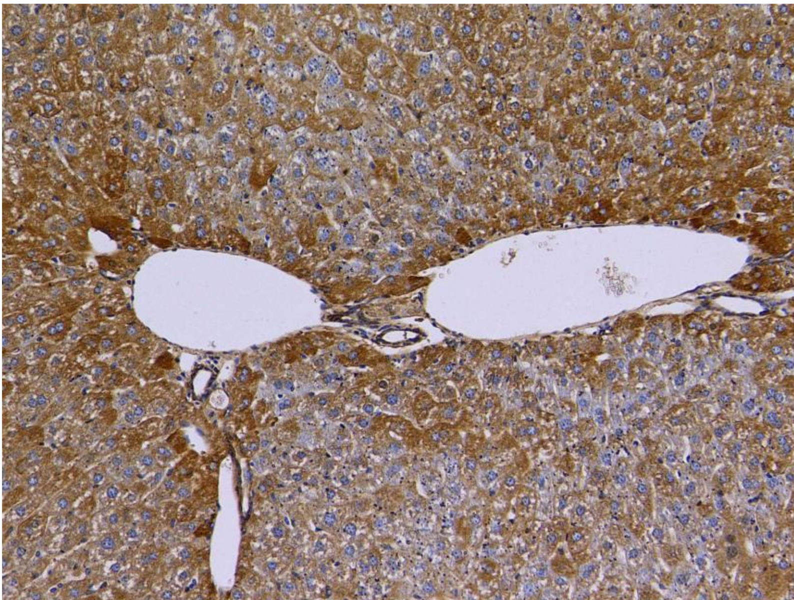
For more information, please visit our web site http://ruo.mbl.co.jp/.

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

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% H_2O_2 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 suggested 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

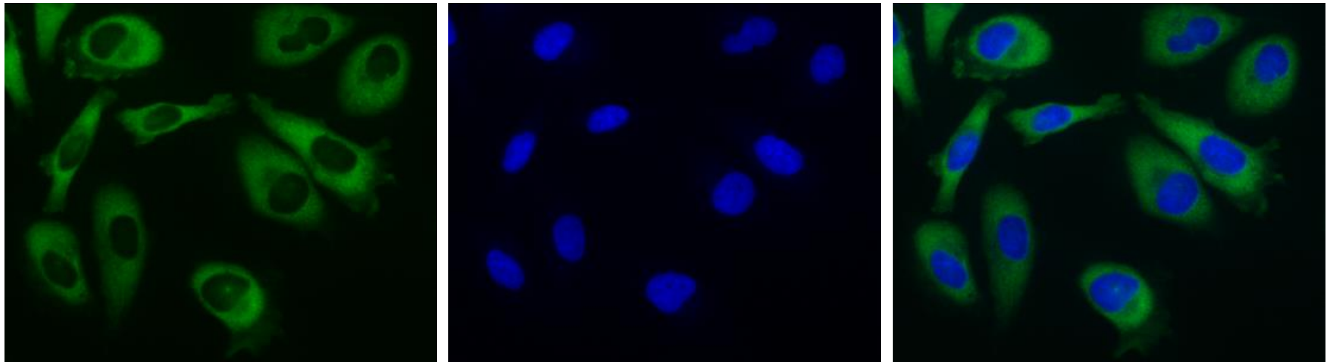
Brown: Anti-1-methyladenosine (m^1A) mAb (D345-3)
Blue: Hematoxylin

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 CO_2 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 (m^1A) mAb (D345-3)
Blue: Hoechst33342

RNA immunoprecipitation

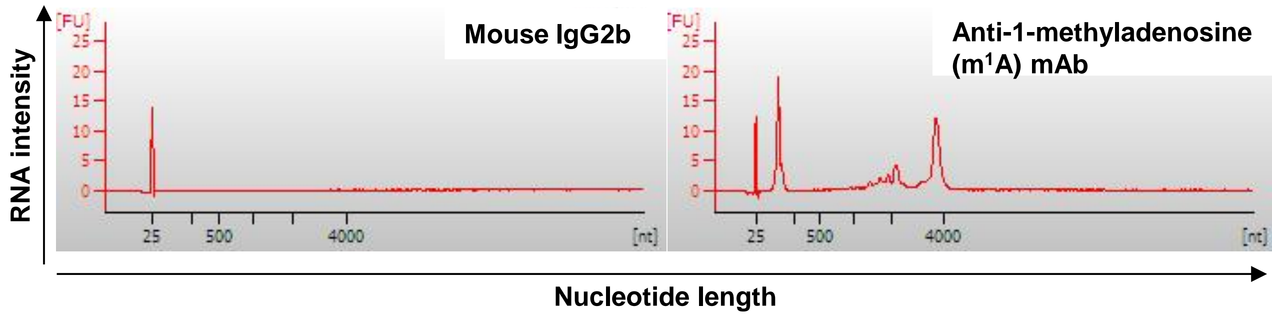
[Material Preparation]

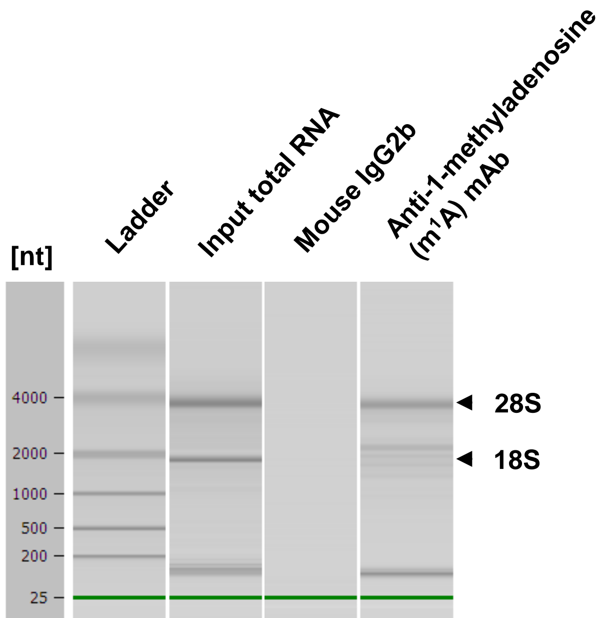
1. *RNA-IP Buffer* [mi-Lysis Buffer (component of RN1005) containing 50 U/mL RNase inhibitor and 1.5 mM DTT]
   Before using the RNA-IP Buffer, RNase inhibitor and DTT are added to mi-Lysis Buffer at the appropriate concentration.
2. *Wash Buffer* [mi-Wash Buffer (component of RN1005) containing 1.5 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 µL of 50% protein G agarose beads slurry resuspended in nuclease-free PBS with 600 µL of Wash Buffer, and then add Mouse IgG2b (isotype control) (MBL; code no. M077-3) or Anti-1-methyladenosine (m^1A) 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 µg 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 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 or below 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 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 µL of ice-cold 70% ethanol and dry up the pellet for 5-15 min.
10) Dissolve the pellet in 20 µL 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^1A) mAb	320.0