

RIP-Certified Antibody

RiboCluster Profiler™

Anti-2,2,7-trimethylguanosine (m₃G/TMG) mAb

CODE No.	RN019M
CLONALITY	Monoclonal
CLONE	235-1
ISOTYPE	Mouse IgG2a κ
QUANTITY	200 μL, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	Carrier protein-conjugated trimethylguanosine (m ₃ G/TMG)
REACTIVITY	This clone reacts with trimethylguanosine (m ₃ G/TMG) cap structure of small RNA, such as snRNA and snoRNA.
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

<u>RNA immunoprecipitation</u>	10 μg/sample
<u>Immunocytochemistry</u>	0.5-1 μg/mL

REFERENCES

- 1) Tsuiji, H., *et al.*, *EMBO Mol. Med.* **5**, 221-234 (2013)
- 2) Yedavalli, V. S. and Jeang, K. T., *PNAS* **107**, 14787-14792 (2010)
- 3) Fox, A. H., *et al.*, *Curr. Biol.* **12**, 13-25 (2002)
- 4) Nagai, K., *et al.*, *Biochem. Soc. Trans.* **29**, 15-26 (2001)
- 5) Nottrott, S., *et al.*, *EMBO J.* **18**, 6119-6133 (1999)

RELATED PRODUCTS

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

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* (MBL, code no. RN1005). Please also refer to the protocol packaged in the RIP-Assay Kit *for microRNA*.

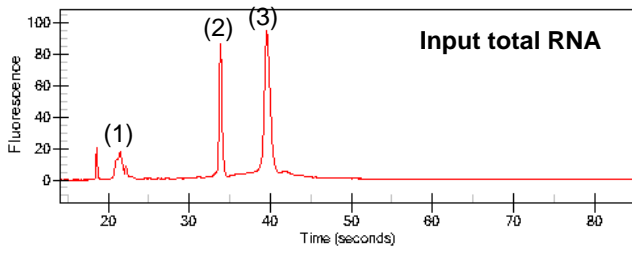
[Material Preparation]

1. ***RNA-IP Buffer*** [mi-Lysis Buffer (component of RN1005) containing 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 mi-Wash Buffer (component of RN1005), and then add Mouse IgG2a (isotype control) (MBL, code no. M076-3) or Anti-2,2,7-trimethylguanosine (m_3 G/TMG) mAb (MBL, code no. RN019M) at the concentration suggested in the **APPLICATIONS**. Incubate with gentle agitation overnight at 4°C.
 - B) Wash the beads once with ice-cold mi-Lysis Buffer (component of RN1005) containing 1.5 mM DTT (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. 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.

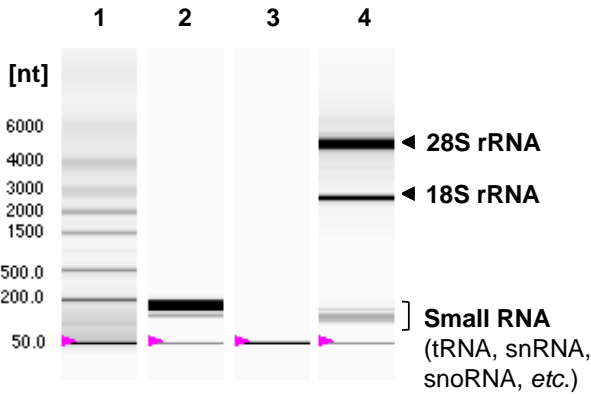
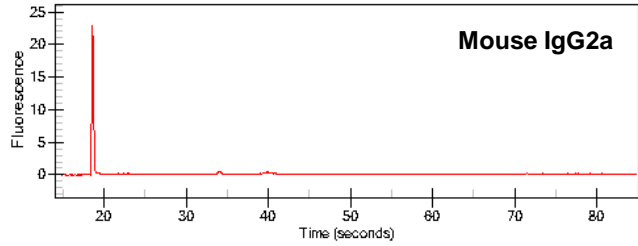
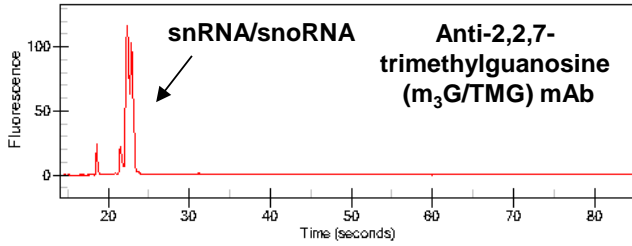
[Protocol (RNA isolation; 2-step method in RN1005)]

- 1) Add 40 μ g of input total RNA and 500 μ 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 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 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 for 20 min. Centrifuge the tube at 12,000 x g for 10 min. at 4°C.
- 9) Wash the pellet twice with 500 μ L of ice-cold 70% ethanol and dry up the pellet for 5-15 min.
- 10) Dissolve the pellets 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 Experion (Bio-Rad).

(Positive control for RNA immunoprecipitation; HEK293T total RNA)



- (1) Small RNA (tRNA, snRNA, snoRNA, etc.)
- (2) 18S rRNA
- (3) 28S rRNA



- Lane 1: Ladder
- Lane 2: Anti-2,2,7-trimethylguanosine (m_3G/TMG) mAb
- Lane 3: Mouse IgG2a
- Lane 4: Input total RNA

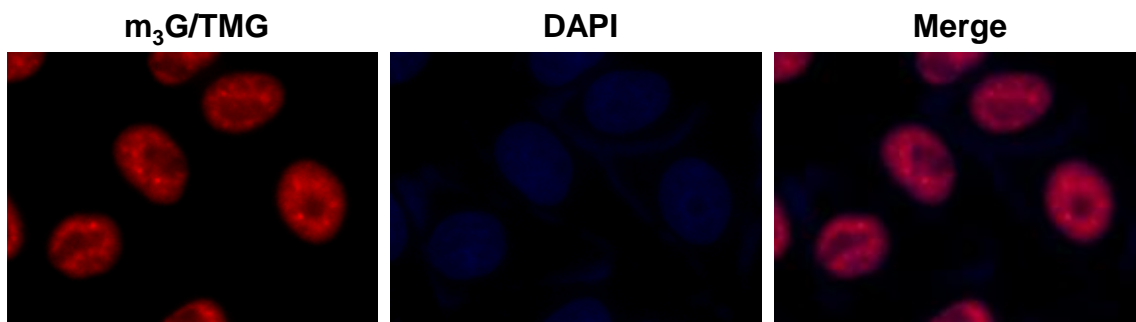
Analysis of RNA with Experion

Average of the RNA Quantitiy (n=2)	
Antibody	RNA (ng)
Anti-2,2,7-trimethylguanosine (m_3G/TMG) mAb	436.0
Mouse IgG2a	84.3

Immunocytochemistry

- 1) Spread the cells on a glass slide, then incubate in a CO₂ incubator overnight.
- 2) Remove the culture supernatant by careful aspiration.
- 3) Wash the slide 3 times with PBS.
- 4) Fix the cells with 4% paraformaldehyde/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/PBS for 10 min. at room temperature.
- 7) Wash the slide 3 times with PBS.
- 8) Block the cells with blocking buffer (1% BSA/0.05% Tween-20/PBS) for 1 hr. at room temperature.
- 9) Tip off the blocking buffer and incubate the cells with the primary antibody diluted with PBS as suggested in the **APPLICATIONS** for 1 hr. at room temperature.
- 10) Wash the slide 3 times with 0.05% Tween-20/PBS.
- 11) Incubate the cells with 1:200 Alexa Fluor® 594 Goat Anti-Mouse IgG (Thermo Fisher Scientific, code no. A-11032) diluted with PBS for 1 hr. at room temperature in dark chamber.
- 12) Wash the slide 3 times with 0.05% Tween-20/PBS.
- 13) Counterstain with DAPI for 2 min. at room temperature.
- 14) Wash the slide with PBS.
- 15) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; HeLa)



Immunocytochemical detection of m₃G/TMG in HeLa

Red: Anti-2,2,7-trimethylguanosine (m₃G/TMG) mAb (RN019M)
Blue: DAPI