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

MBL

RIP-Certified Antibody

RiboCluster Profiler™

Anti-2,2,7-trimethylguanosine (m_3G/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_3G/TMG)
REACTIVITY	This clone reacts with trimethylguanosine (m_3G/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

RNA immunoprecipitation	10 µg/sample
Immunocytochemistry	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*.

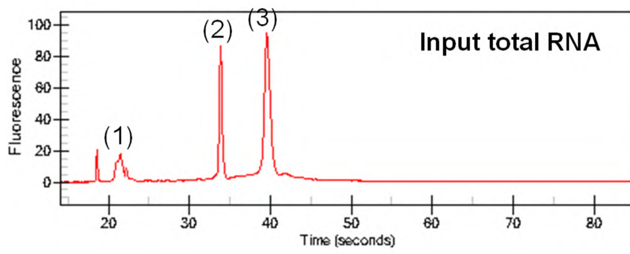
[Material Preparation]

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 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 Mouse IgG2a (isotype control) (MBL, code no. M076-3) or Anti-2,2,7-trimethylguanosine (m_3G/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.

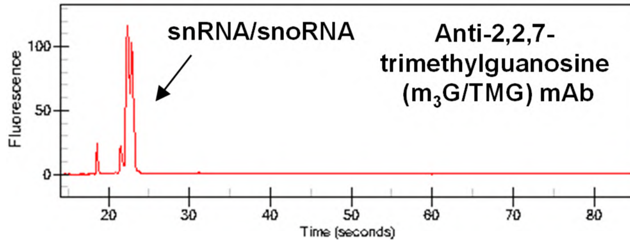
[Protocol (RNA isolation; 2-step method in RN1005)]

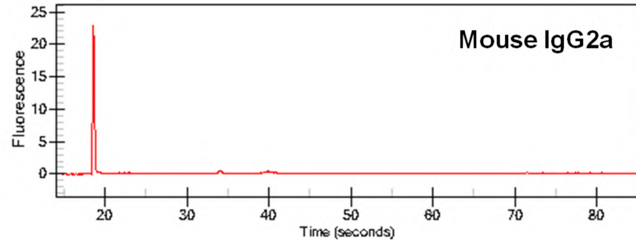
1) Add 40 µg of input total RNA and 500 µL of RNA-IP Buffer into the tube containing antibody conjugated beads, then incubate with gentle agitation for 3 hr. at 4°C.
2) Wash the beads 4 times with 1 mL of Wash Buffer (centrifuge the tube at 2,000 x g for 1 min.).
3) Add 250 µL of Master mix solution (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 twice 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).

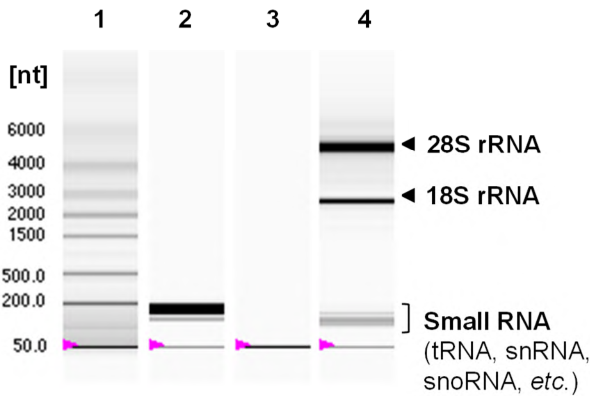
(Positive control for RNA immunoprecipitation; HEK293T total RNA)



(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

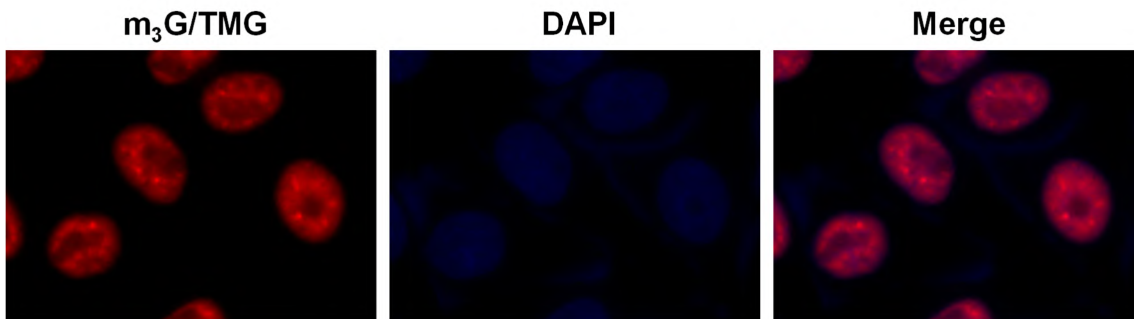
Analysis of RNA with Experion

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 CO_2 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 m_3G/TMG in HeLa
Red: Anti-2,2,7-trimethylguanosine (m_3G/TMG) mAb (RN019M)
Blue: DAPI