

RiboCluster Profiler™

Anti-7-methylguanosine (m⁷G) mAb

CODE No.	RN017M
CLONALITY	Monoclonal
CLONE	4141-13
ISOTYPE	Mouse IgG2a κ
QUANTITY	200 μL, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	Carrier protein-conjugated 7-methylguanosine (m ⁷ G)-Cap analogue
REACTIVITY	This clone reacts with both 5'-terminal and internal 7-methylguanosine (m ⁷ G) in RNA.
FORMURATION	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>Dot blotting</u>	1 μg/mL
<u>RNA immunoprecipitation</u>	10 μg/sample
<u>Immunocytochemistry</u>	Can be used.
<u>RNA EISA</u>	Can be used.

REFERENCES

- 1) Zorbas, C., *et al.*, *Mol. Biol. Cell.* **26**, 2080-2095 (2015)
- 2) Filonava, L., *et al.*, *Genome Biol.* **16**, 216 (2015)
- 3) Chen, P., *et al.*, *BMC Plant Biol.* **10**, 201 (2010)
- 4) Cubrilo, S., *et al.*, *RNA* **15**, 1492-1497 (2009)
- 5) Cowling, V. H., *Biochem. J.* **425**, 295-302 (2009)
- 6) Maden, B. E. and Salim, M., *J. Mol. Biol.* **88**, 133-152 (1974)

For more information, please visit our web site <http://ruo.mbl.co.jp/je/rip-assay/>

LICENSING OPPORTUNITY: The RIP-Assay uses patented technology (US patent No. 6,635,422, US patent No. 7,504,210, JP patent No. 5,002,105) of Ribonomics, Inc. MBL manufactures and distributes this product under license from Ribonomics, Inc. Researchers may use this product for their own research. Researchers are not allowed to use this product or RIP-Assay technology for commercial purpose without a license. For commercial use, please contact us for licensing opportunities at RIP@mbi.co.jp

RELATED PRODUCTS

Antibodies

- RN017M Anti-7-methylguanosine (m⁷G) mAb (4141-13)
RN016M Anti-7-methylguanosine (m⁷G)-Cap mAb (150-15)
RN019M Anti-2,2,7-trimethylguanosine (m₃G/TMG) mAb (235-1)
RN124PW Anti-RNMT (Human) pAb
RN126PW Anti-AHCY (SAHH) pAb
D168-3 Anti-Importin α 1 (Rch1) mAb (1A6)
RN103PW Anti-NCBP1 (CBP80) pAb
RN105PW Anti-PARN pAb
RN109PW Anti-XRN1 (Human) pAb
RN001P Anti-EIF4E pAb
RN006M Anti-EIF4E mAb (C107-3-5)
RN002P Anti-EIF4G1 (Human) pAb
RN003P Anti-EIF4G2 pAb
RN131P Anti-N⁶-methyladenosine (m⁶A) pAb
RN121PW Anti-FTO (Human) pAb
RN122PW Anti-ALKBH5 pAb
RN123PW Anti-YTHDF2 pAb
RN052PW Anti-HNRNPC pAb
D216-3 Anti-hnRNP-A2/B1 mAb (C20308)
D345-3 Anti-1-methyladenosine (m¹A) mAb (AMA-2)
RN128PW Anti-TRMT6 (Human) pAb
RN130PW Anti-TRMT61A (Human) pAb
D346-3 Anti-5-methylcytidine (m⁵C) mAb (FMC-9)
RN127PW Anti-NSUN2 (Human) pAb
M218-3 Anti-5-hydroxymethylcytosine (5hmC) mAb (1G10)
PM077 Anti-5-hydroxymethylcytosine (5hmC) pAb
M229-3 Anti-MECP2 mAb (2-8)
RN125PW Anti-HENMT1 pAb
D347-3 Anti-Pseudouridine mAb (APU-6)
MI-11-3 Anti-Bromodeoxyuridine mAb (2B1)
MI-11-5 Anti-Bromodeoxyuridine mAb-PE (2B1)
M227-3 Anti-Digoxigenin (DIG) mAb (8-10)
M228-3 Anti-FITC mAb (47-11)
- M076-3 Mouse IgG2a (isotype control) (6H3)

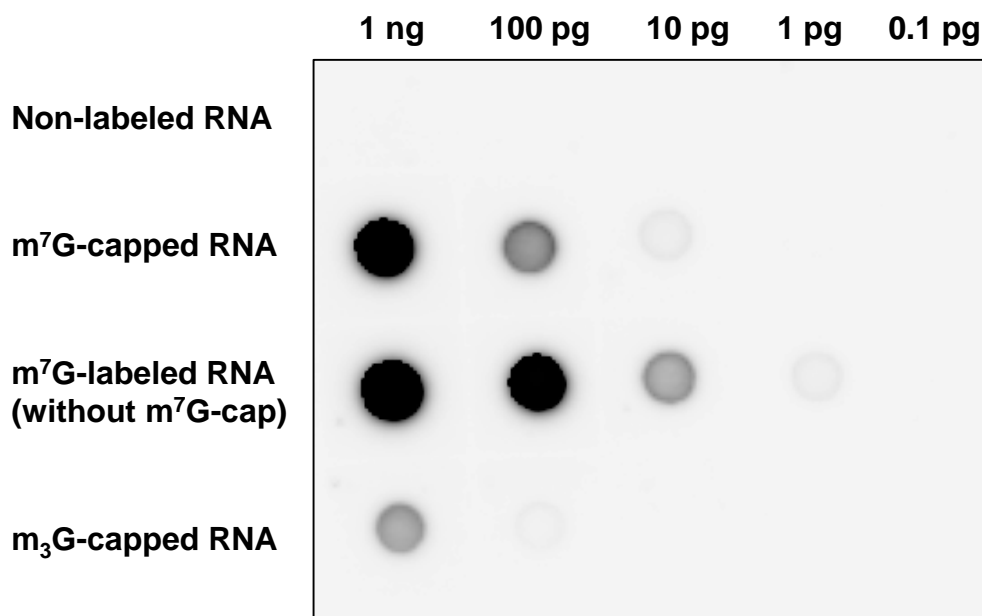
Kit

- RN1005 RIP-Assay Kit *for microRNA*

Dot blotting

Dot blotting was performed using DIG Wash and Block Buffer Set (Sigma-Aldrich; code no. 11585762001). For more information, please contact Sigma-Aldrich Co. LLC.

- 1) Sample preparation:
 - a) Prepare RNA samples by appropriate method (e.g., m⁷G-capped RNA by *in vitro* transcription).
 - b) Heat the RNA samples at 80°C for 2 min., then quench at 4°C for 5 min.
- 2) Blot 1 µL of different concentrations of the RNA samples onto a nitrocellulose membrane.
- 3) Cross-link the RNA samples using UV illuminator.
- 4) To reduce nonspecific binding, soak the membrane in Blocking Buffer for 30 min. at room temperature.
- 5) Incubate the membrane with primary antibody diluted with Blocking Buffer as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 6) Wash the membrane with Wash Buffer (15 min. x 2 times).
- 7) Incubate the membrane with 1:5,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with Blocking Buffer for 1 hr. at room temperature.
- 8) Wash the membrane with Wash Buffer (15 min. x 2 times).
- 9) Wash the membrane with Wash Buffer (3 min. x 1 time).
- 10) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 11) Expose for 40 sec. with ImageQuant LAS 4000 imaging system (Fujifilm). The condition for exposure and development may vary.



Dot blot analysis of m⁷G-capped RNA

Sample: *In vitro* transcribed RNA from full-length of RN7SK RNA (RefSeq ID: NR_001445)

Immunoblotted with Anti-7-methylguanosine (m⁷G) mAb (RN017M)

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*.

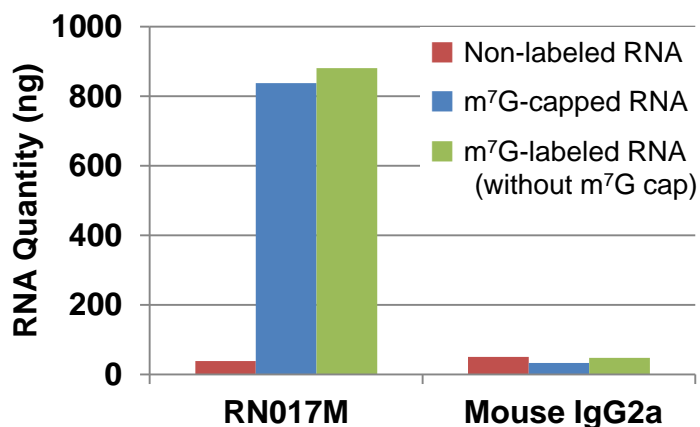
[Material Preparation]

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 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-7-methylguanosine (m^7G) mAb (RN017M) 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)]

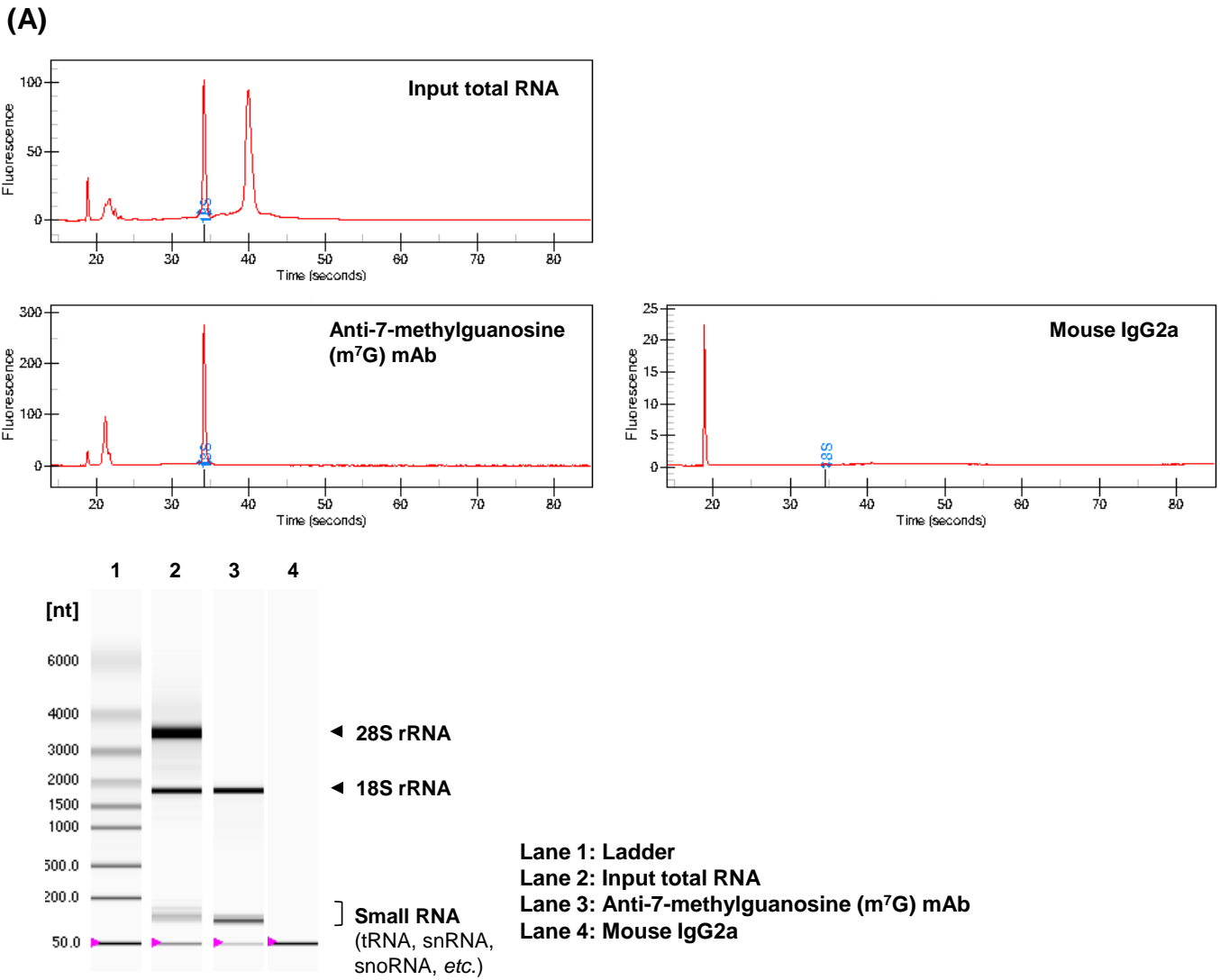
- 1) Add 40 μ g of input total RNA and 500 μ L of RNA-IP Buffer into the tube containing antibody conjugated beads, then incubate with gentle agitation for 3 hr. at 4°C.
- 2) Wash the beads 4 times with 1 mL of Wash Buffer (centrifuge the tube at 2,000 x g for 1 min.).
- 3) Add 250 μ L of Master mix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



RNA immunoprecipitation from *in vitro* transcribed RNA

Sample: 2 μ g of *in vitro* transcribed RNA
from full-length of RN7SK RNA
(RefSeq ID: NR_001445)



(B)

Average of the RNA Quantity (n=2)	
Antibody	RNA (ng)
Anti-7-methylguanosine (m ⁷ G) mAb	921.0
Mouse IgG2a	42.2

RNA immunoprecipitation from HEK293T total RNA

- (A) Characterization of isolated RNA with Experion
(B) Quantification of isolated RNA with NanoDrop