

RiboCluster Profiler™

Anti-7-methylguanosine (m⁷G)-Cap mAb

CODE No.	RN016M
CLONALITY	Monoclonal
CLONE	150-15
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 5'-terminal 7-methylguanosine (m ⁷ G) cap structure of RNA and partially cross-reacts with m ⁷ G within RNA.
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>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) Ramanathan, A., <i>et al.</i> , <i>Nucleic Acids Res.</i> 44 , 7511-7526 (2016)
	2) Cowling, V. H., <i>Biochem. J.</i> 425 , 295-302 (2009)
	3) Rottman, F., <i>et al.</i> , <i>Cell</i> 3 , 197-199 (1974)

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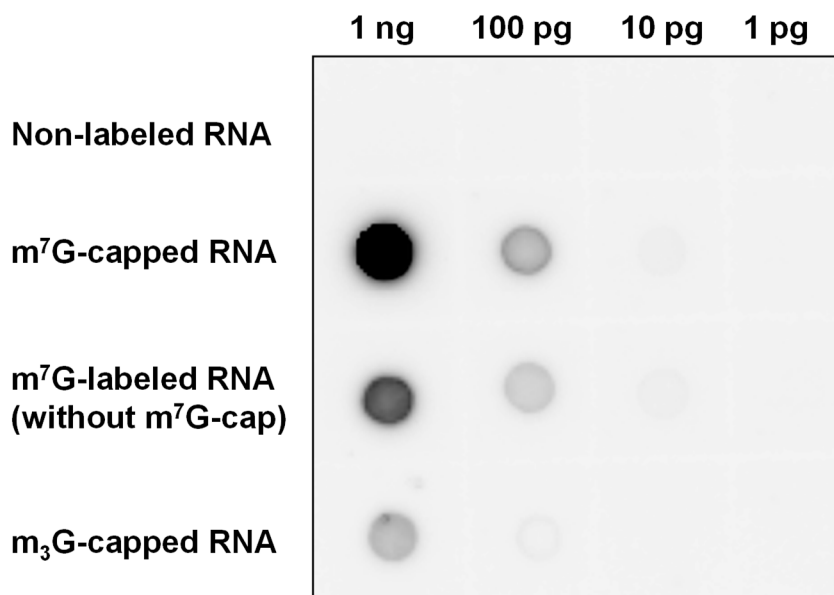
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The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

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).
- 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).
- 9) Wash the membrane with Wash Buffer (3 min. x 1).
- 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 2 min. 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)-Cap mAb (MBL, code no. RN016M)

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*.

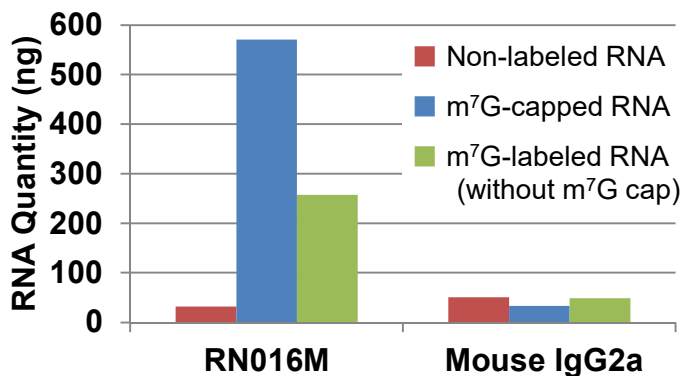
[Material Preparation]

1. ***RNA-IP Buffer (-)*** [mi-Lysis Buffer (component of RN1005) containing 1.5 mM DTT]
Before using RNA-IP Buffer (-), DTT is added to mi-Lysis Buffer at the appropriate concentration.
2. ***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.
3. ***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.
4. 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 (m⁷G)-Cap mAb (MBL, code no. RN016M) at the concentration suggested in the **APPLICATIONS**. Incubate with gentle agitation overnight at 4°C.
 - B) Wash the beads once with ice-cold RNA-IP Buffer (-).
 - C) Carefully discard the supernatant using a pipettor without disturbing the beads and incubate at 4°C until just before use.
5. 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).

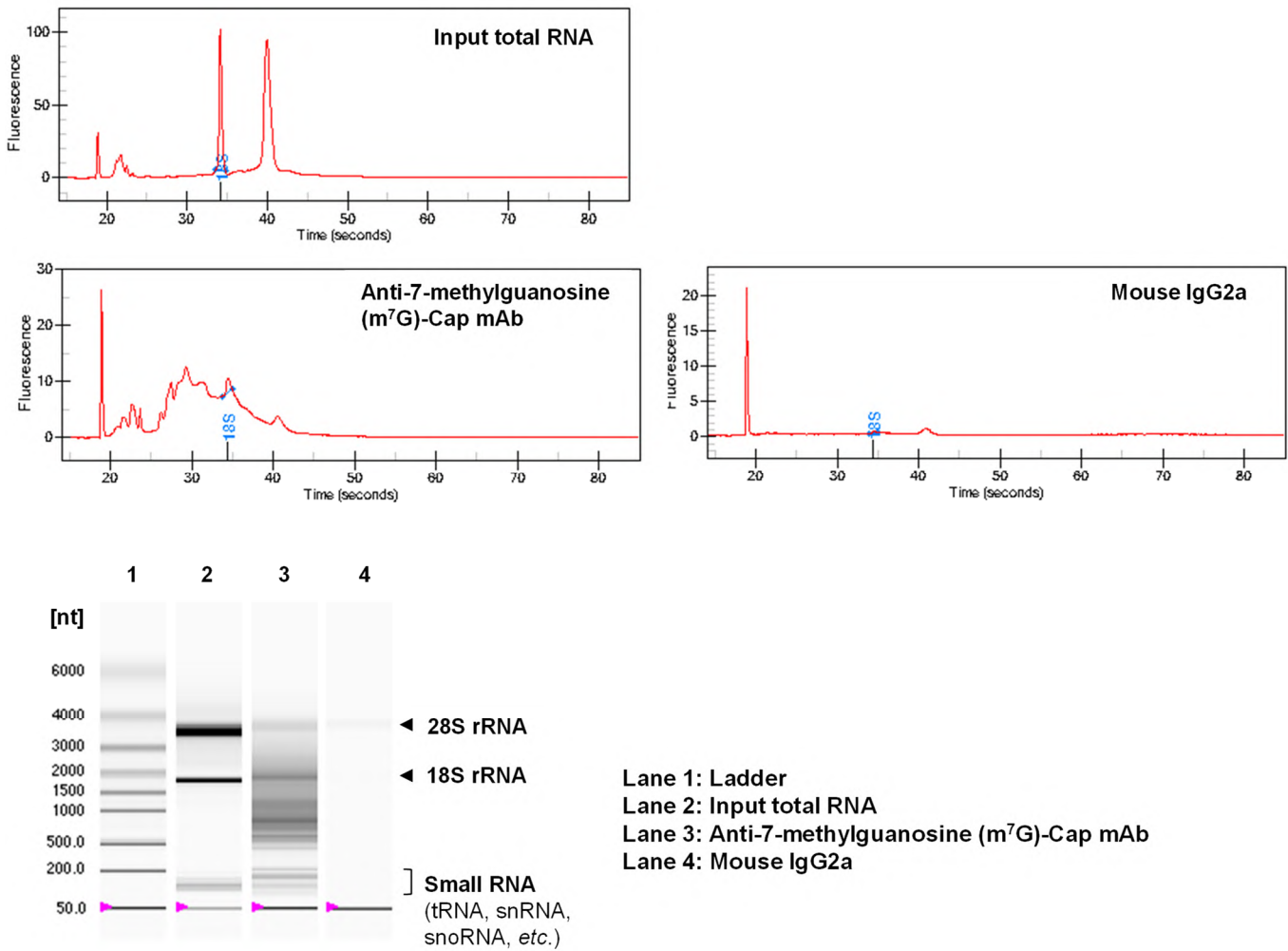
(Positive control for RNA immunoprecipitation; HEK293T total RNA)



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)

(A)



(B)

Average of the RNA Quantity (n=2)	
Antibody	RNA (ng)
Anti-7-methylguanosine (m ⁷ G)-Cap mAb	132.1
Mouse IgG2a	42.2

RNA immunoprecipitation from HEK293T total RNA

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