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RiboCluster Profiler™

Anti-7-methylguanosine (m^7G)-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^7G)-Cap analogue
REACTIVITY	This clone reacts with 5′-terminal 7-methylguanosine (m^7G) cap structure of RNA and partially cross-reacts with m^7G 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

Dot blotting	1 µg/mL
RNA immunoprecipitation	10 µg/sample
Immunocytochemistry	Can be used.
RNA EISA	Can be used.

REFERENCES

1) Ramanathan, A., *et al.*, *Nucleic Acids Res.* **44**, 7511-7526 (2016)
2) Cowling, V. H., *Biochem. J.* **425**, 295-302 (2009)
3) Rottman, F., *et al.*, *Cell* **3**, 197-199 (1974)

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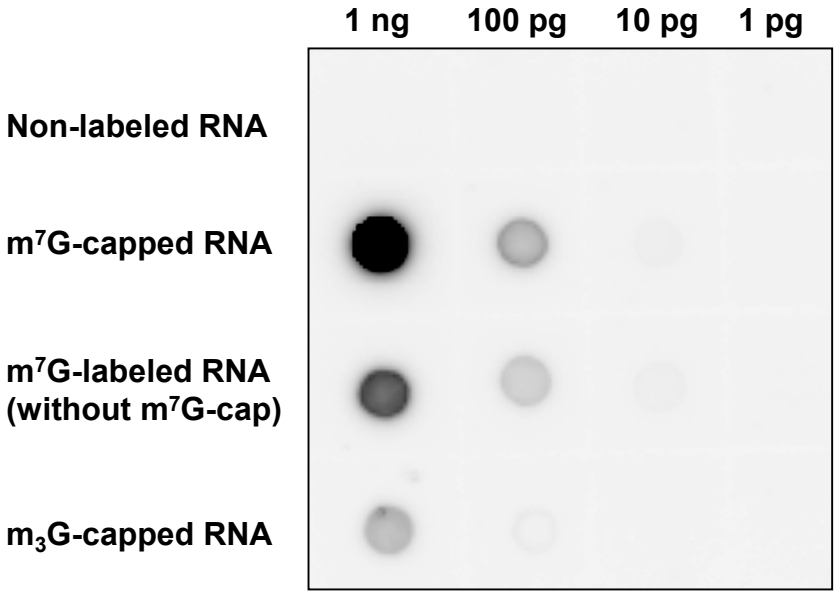
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

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., m^7G-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 m^7G-capped RNA

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

Immunoblotted with Anti-7-methylguanosine (m^7G)-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*.

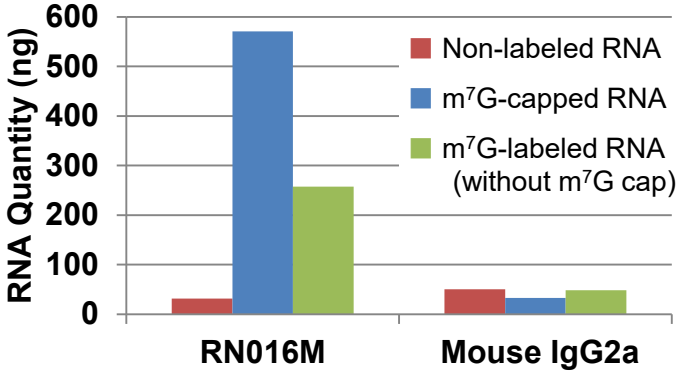
[Material Preparation]

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 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-7-methylguanosine (m^7G)-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 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).

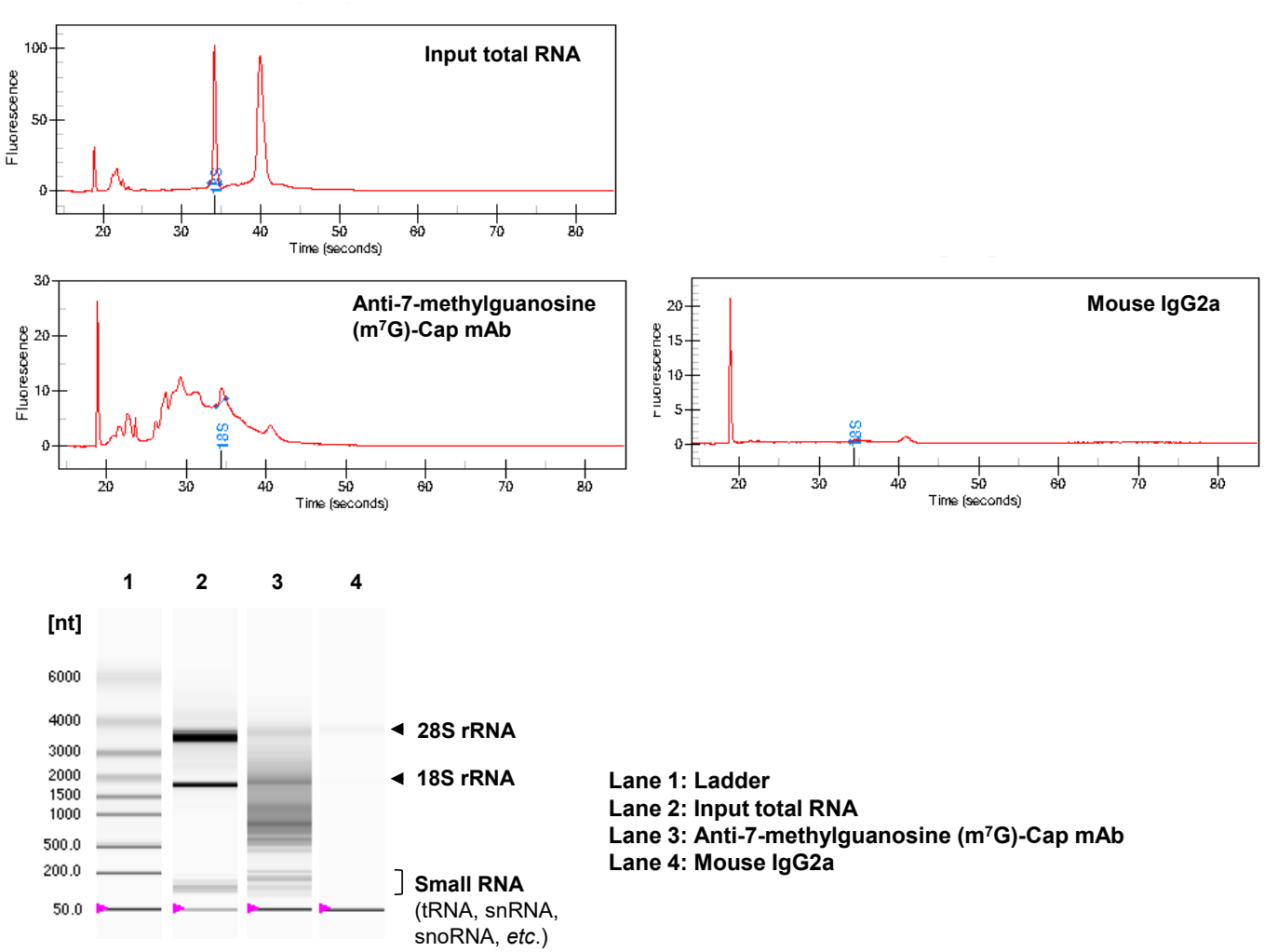
(Positive control for RNA immunoprecipitation; HEK293T total RNA)



RNA immunoprecipitation from in vitro transcribed RNA

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

(A)



(B)

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

RNA immunoprecipitation from HEK293T total RNA

(A) Characerization of isolated RNA with Experion
(B) Quantification of isolated RNA with NanoDrop