

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



Anti-Digoxigenin (DIG) mAb

CODE No.	M227-3
CLONALITY	Monoclonal
CLONE	8-10
ISOTYPE	Mouse IgG1 κ
QUANTITY	100 μ L, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	KLH-conjugated Digoxigenin (DIG)
FORMULATION	PBS containing 50% glycerol. 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 μ g/mL for chemiluminescence detection system
<u>Northern blotting</u>	Can be used.
<u>RNA Fluorescence <i>in situ</i> hybridization</u>	Can be used.
<u>RNA Immunoprecipitation</u>	Can be used.
<u>RNA ELISA</u>	1 μ g/mL
<u>Western blotting</u>	1 μ g/mL for chemiluminescence detection system

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



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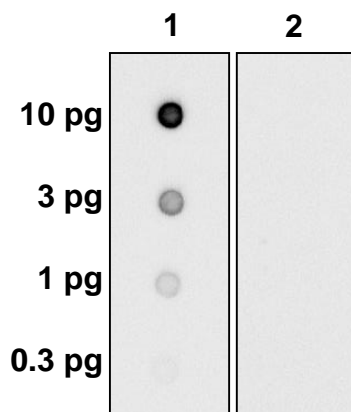
RELATED PRODUCTS

M227-3	Anti-Digoxigenin (DIG) mAb (8-10)
M228-3	Anti-FITC mAb (47-11)
MI-11-3	Anti-Bromodeoxyuridine mAb (2B1)
MI-11-5	Anti-Bromodeoxyuridine mAb-PE (2B1)
D345-3	Anti-1-methyladenosine (m ¹ A) mAb (AMA-2)
RN131P	Anti-N ⁶ -methyladenosine (m ⁶ A) pAb
D347-3	Anti-Pseudouridine mAb (APU-6)
RN016M	Anti-7-methylguanosine (m ⁷ G)-Cap mAb (150-15)
RN017M	Anti-7-methylguanosine (m ⁷ G) mAb (4141-13)
RN019M	Anti-2,2,7-trimethylguanosine (m ₃ G/TMG) mAb (235-1)
D346-3	Anti-5-methylcytidine (m ⁵ C) mAb (FMC-9)
M218-3	Anti-5-hydroxymethylcytosine (5hmC) mAb (1G10)
PM077	Anti-5-hydroxymethylcytosine (5hmC) pAb
PM098	Anti-Inosine pAb
M075-3	Mouse IgG1 (isotype control) (2E12)

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., Digoxigenin (DIG) labeling 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 3 min. with ImageQuant LAS 4000 imaging system (Fujifilm). The condition for exposure and development may vary.



Dot blot analysis of Digoxigenin (DIG)-labeled RNA

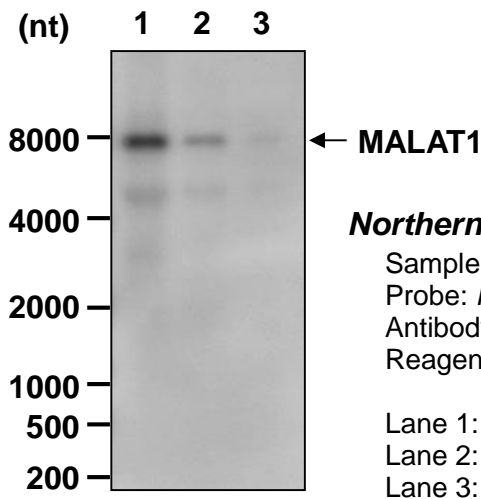
Sample: DIG-labeled RNA synthesized by *in vitro* transcription from *lacZ*-encoding cDNA
(RefSeq ID: NC_007779.1, region 363130-364149)

<Immunoblot>

Lane 1: Anti-Digoxigenin (DIG) mAb (M227-3)

Lane 2: Mouse IgG1 (isotype control) (M075-3)

Northern blotting

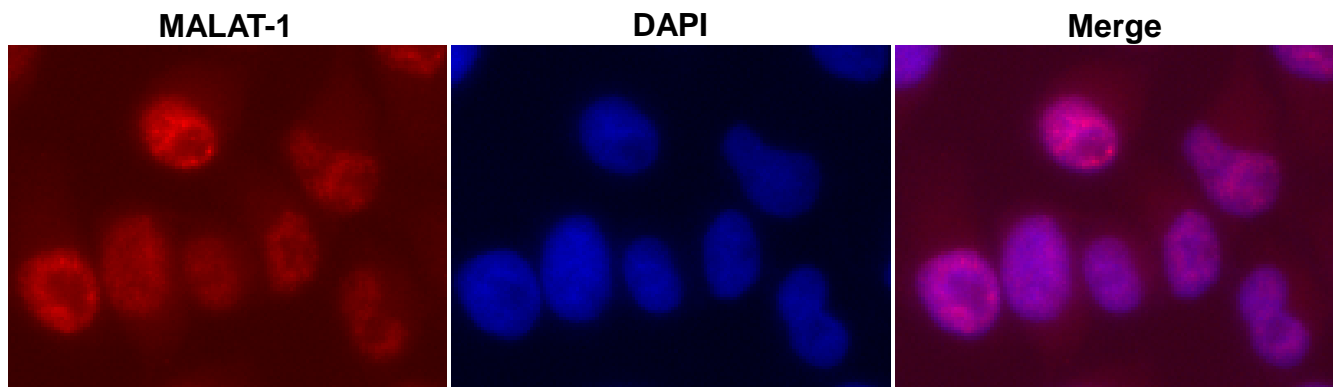


Northern blot analysis of MALAT1 ncRNA

Sample: Total RNA extracted from HEK293T cells
Probe: MALAT1 ncRNA (RefSeq ID: NR_002819.3, region 6641-7113)
Antibody: Anti-Digoxigenin (DIG) mAb (M227-3), 1 µg/mL
Reagent: DIG Wash and Block Buffer Set (Sigma-Aldrich; code no. 11585762001)

Lane 1: 500 ng of total RNA
Lane 2: 100 ng of total RNA
Lane 3: 20 ng of total RNA

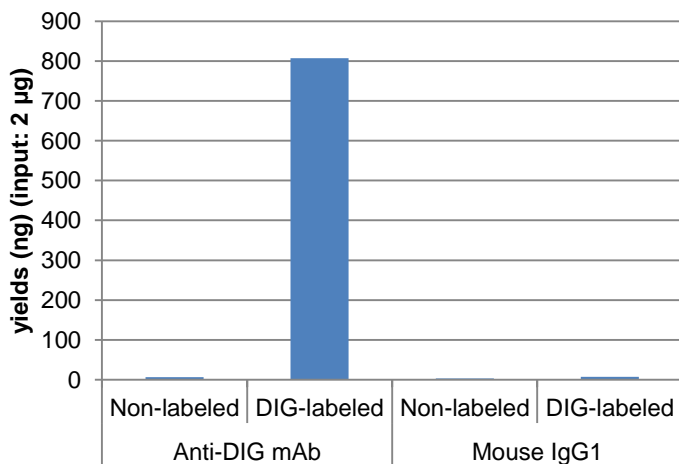
RNA Fluorescence *in situ* hybridization (RNA FISH)



Fluorescence *in situ* hybridization of MALAT1 ncRNA

Cells: HeLa
Probe: MALAT1 ncRNA (RefSeq ID: NR_002819.3, region 6641-7113)
Antibody: Anti-Digoxigenin (DIG) mAb (M227-3), 1 µg/mL

RNA immunoprecipitation

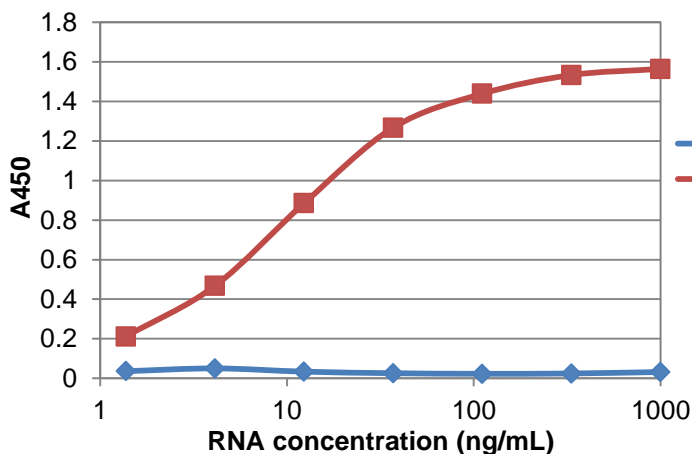


RNA immunoprecipitation of DIG-labeled RNA

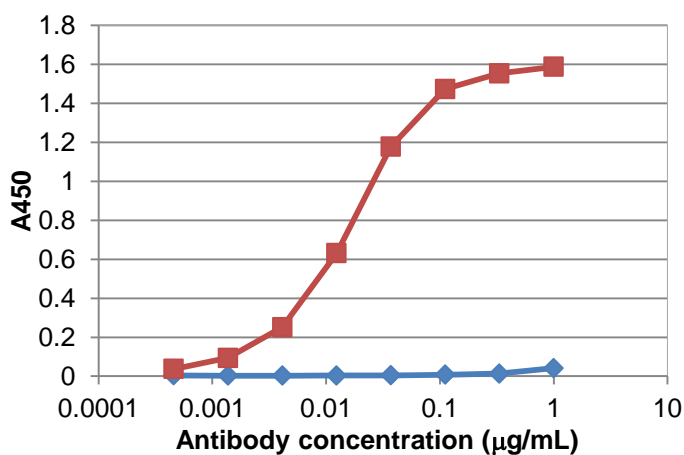
Sample: DIG-labeled RNA synthesized by *in vitro* transcription from *lacZ*-encoding cDNA (RefSeq ID: NC_007779.1, region 363130-364149)
Antibody: Anti-Digoxigenin (DIG) mAb (M227-3), 10 µg
Mouse IgG1 (isotype control) (M075-3), 10 µg

RNA ELISA

- 1) Add 50 μL /well of Poly-L-lysine solution (Sigma; code no. P4832-50ML) diluted with nuclease-free water to the 96-well plate. Incubate for 1 hr. at 37°C.
- 2) Wash the plate 3 times with nuclease-free water.
- 3) Sample preparation:
 - a) Prepare RNA samples by appropriate method (e.g., Digoxigenin (DIG) labeling RNA by *in vitro* transcription).
 - b) Heat RNA samples diluted with nuclease-free water at 80°C for 2 min., then quench at 4°C for 5 min.
- 4) Add 50 μL /well of RNA samples to the Poly-L-lysine-coated 96-well plate. Incubate for 1 hr. at room temperature.
- 5) Wash the plate 3 times with PBS.
- 6) Add 150 μL /well of Blocking Buffer [Blocking Reagent-N101 (NOF CORPORATION; code no. S410-03012), 5-fold dilution with nuclease-free water]. Incubate for 1 hr. at room temperature.
- 7) Wash the plate 3 times with PBS-T [0.05% Tween-20 in PBS].
- 8) Add 50 μL /well of primary antibody diluted with PBS as suggested in the **APPLICATIONS**. Incubate for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 9) Wash the plate 3 times with PBS-T.
- 10) Add 50 μL /well of 1:10,000 Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with PBS. Incubate for 1 hr. at room temperature.
- 11) Wash the plate 3 times with PBS-T.
- 12) Add 50 μL /well of substrate solution (ex. TMB). Incubate for appropriate time at room temperature.
- 13) Add 50 μL /well of stop solution (ex. 1.5 N H_3PO_4 in distilled water.).
- 14) Read absorbance at 450 nm.



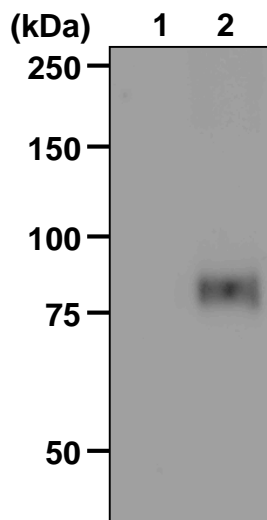
ELISA for measurement of DIG-labeled RNA
Sample: DIG-labeled RNA synthesized by *in vitro* transcription from *lacZ*-encoding cDNA (RefSeq ID: NC_007779.1, region 363130-364149)
Antibody: Anti-Digoxigenin (DIG) mAb (M227-3), 1 $\mu\text{g}/\text{mL}$



ELISA for measurement of DIG-labeled RNA
Sample: DIG-labeled RNA synthesized by *in vitro* transcription from *lacZ*-encoding cDNA (RefSeq ID: NC_007779.1, region 363130-364149), 0.5 $\mu\text{g}/\text{mL}$
Antibody: Anti-Digoxigenin (DIG) mAb (M227-3)

SDS-PAGE & Western blotting

- 1) Boil the sample for 3 min. and centrifuge. Load 10 μ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (7.5% acrylamide) for electrophoresis.
- 2) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer's manual for precise transfer procedure.
- 3) To reduce nonspecific binding, soak the membrane in blocking buffer [PBS containing 1% BSA and 0.05% Tween-20] for 1 hr. at room temperature
- 4) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
- 5) Incubate the membrane with primary antibody diluted with blocking buffer as suggested in the **APPLICATION** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 6) Wash the membrane with PBS-T (5 min. x 3 times).
- 7) Incubate the membrane with 1:5,000 Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with blocking buffer for 30 min. at room temperature.
- 8) Wash the membrane with PBS-T (5 min. x 3 times).
- 9) 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 a plastic wrap.
- 10) Expose for 5 min. with ImageQuant LAS 4000 imaging system (Fujifilm). The condition for exposure and development may vary.



Western blot analysis of Digoxigenin-conjugated protein

Lane 1: BSA, 100 pg

Lane 2: DIG-labeled BSA, 100 pg

Immunoblotted with Anti-Digoxigenin (DIG) mAb (M227-3)