

# Anti-Digoxigenin (DIG) mAb

<b>CODE No.</b>	M227-3
<b>CLONALITY</b>	Monoclonal
<b>CLONE</b>	8-10
<b>ISOTYPE</b>	Mouse IgG1 $\kappa$
<b>QUANTITY</b>	100 $\mu$ L, 1 mg/mL
<b>SOURCE</b>	Purified IgG from hybridoma supernatant
<b>IMMUNOGEN</b>	KLH-conjugated Digoxigenin (DIG)
<b>FORMULATION</b>	PBS containing 50% glycerol. No preservative is contained.
<b>STORAGE</b>	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 $\mu$ g/mL
<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 $\mu$ g/mL
<u>Western blotting</u>	1 $\mu$ g/mL

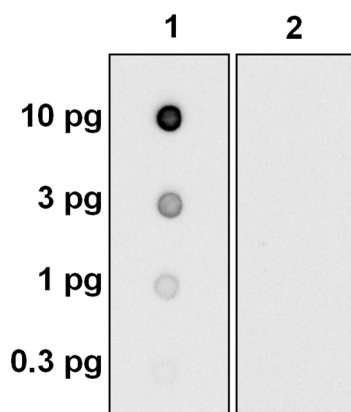
For more information, please visit our website at <https://ruo.mbl.co.jp/>.

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., 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).
- 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 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***

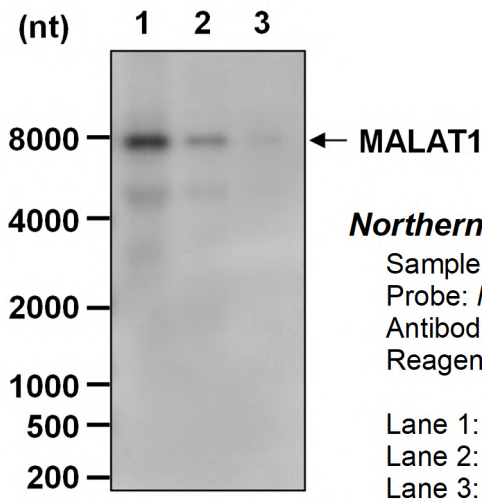
Sample: DIG-labeled RNA synthesized by *in vitro* transcription from *lacZ*-encoding cDNA  
(RefSeq ID: NC\_007779.1, region 363130-364149)

<Immunoblot>

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

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

**Northern blotting**

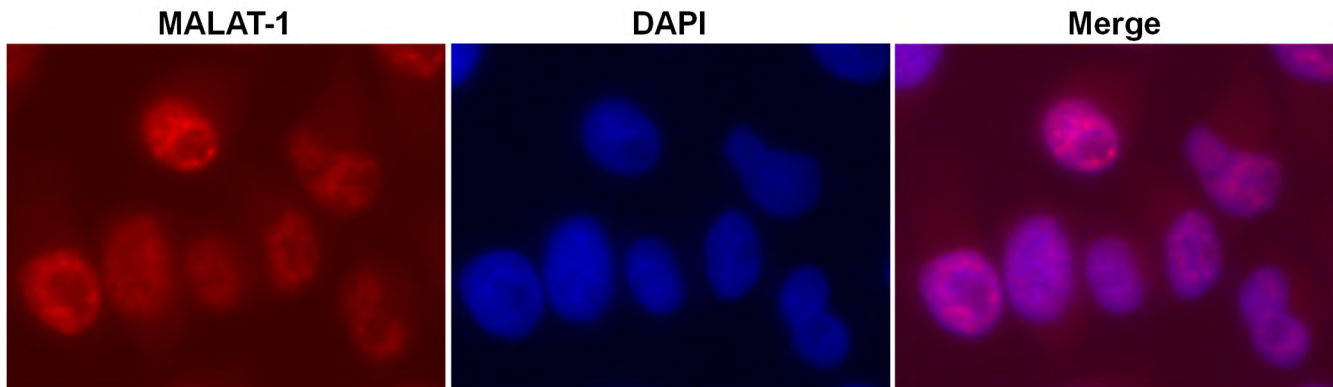


**Northern blot analysis of MALAT1 ncRNA**

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

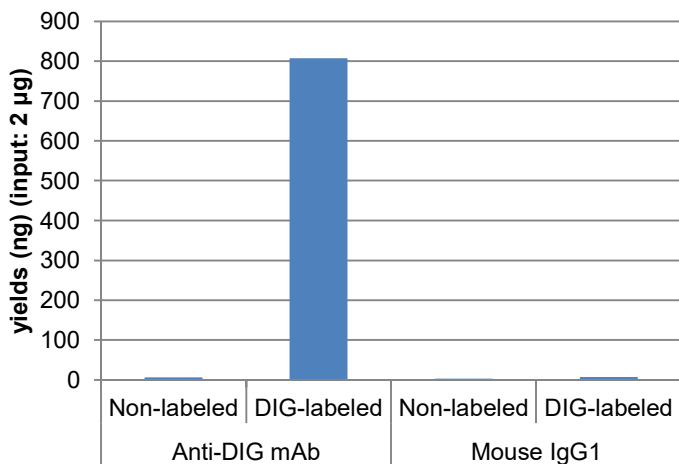
**RNA Fluorescence *in situ* hybridization (RNA FISH)**



**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**

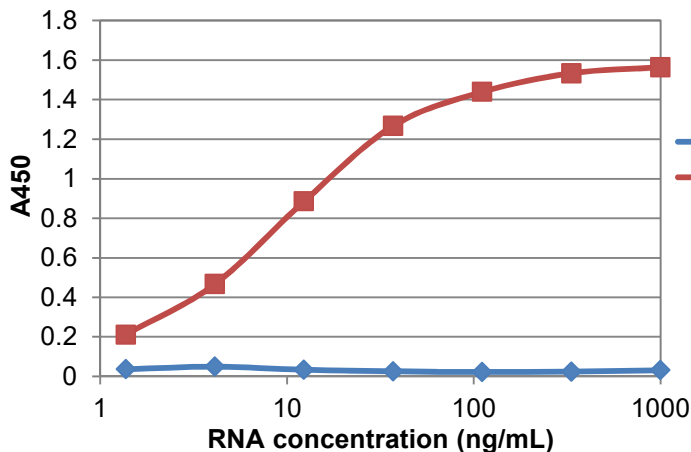


**RNA immunoprecipitation of DIG-labeled RNA**

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

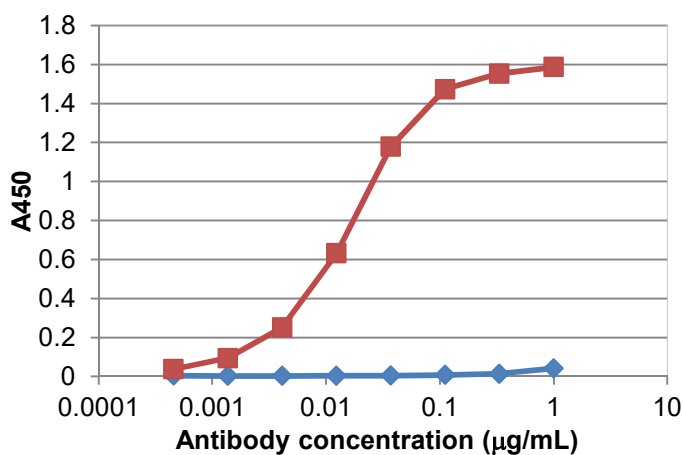
- 1) Add 50  $\mu\text{L}$ /well of Poly-L-lysine solution (Sigma-Aldrich, code no. P4832-50ML) diluted with nuclease-free water to the 96-well plate. Incubate for 1 hr. at 37°C.
- 2) Wash the plate 3 times with nuclease-free water.
- 3) Sample preparation:
  - a) Prepare RNA samples by appropriate method (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  $\mu\text{L}$ /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  $\mu\text{L}$ /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  $\mu\text{L}$ /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  $\mu\text{L}$ /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  $\mu\text{L}$ /well of substrate solution (ex. TMB). Incubate for appropriate time at room temperature.
- 13) Add 50  $\mu\text{L}$ /well of stop solution (ex. 1.5 N  $\text{H}_3\text{PO}_4$  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  $\mu\text{g}/\text{mL}$



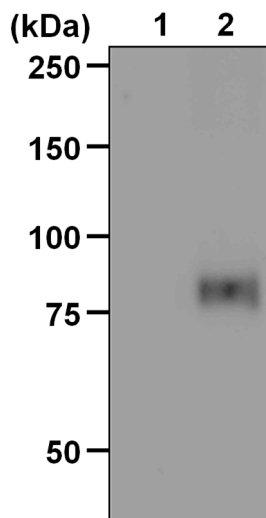
### ELISA for measurement of DIG-labeled RNA

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  $\mu$ 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/cm<sup>2</sup> for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). 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).
- 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).
- 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).
- 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  $\mu$ g

Lane 2: DIG-labeled BSA, 100  $\mu$ g

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