

Anti-FITC mAb

CODE No.	M228-3
CLONALITY	Monoclonal
CLONE	47-11
ISOTYPE	Mouse IgG1 κ
QUANTITY	100 μ L, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	KLH-conjugated FITC
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.
APPLICONS	
<u>Dot blotting</u>	1 μ g/mL
<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
<u>Northern blotting</u>	Can be used.

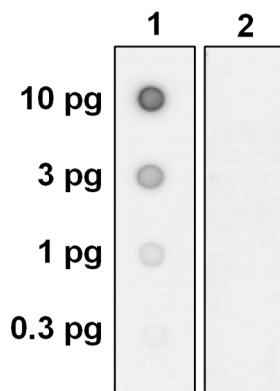
For more information, please visit our web site <https://ruo.mbl.co.jp/>.

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., Fluorescein-labeled 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 10 min. with ImageQuant LAS 4000 imaging system (Fujifilm). The condition for exposure and development may vary.



Dot blotting analysis of FITC-labeled RNA

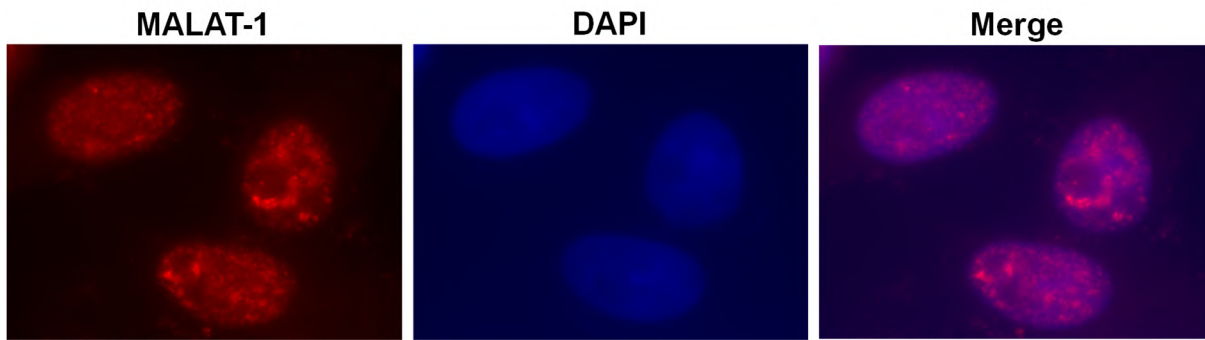
Sample: FITC-labeled RNA synthesized by *in vitro* transcription from *lacZ*-encoding cDNA
(RefSeq ID: NC_007779.1, region 363130-364149)

<Immunoblot>

Lane 1: Anti-FITC mAb (MBL, code no. M228-3)

Lane 2: Mouse IgG1 (isotype control) (MBL, code no. M075-3)

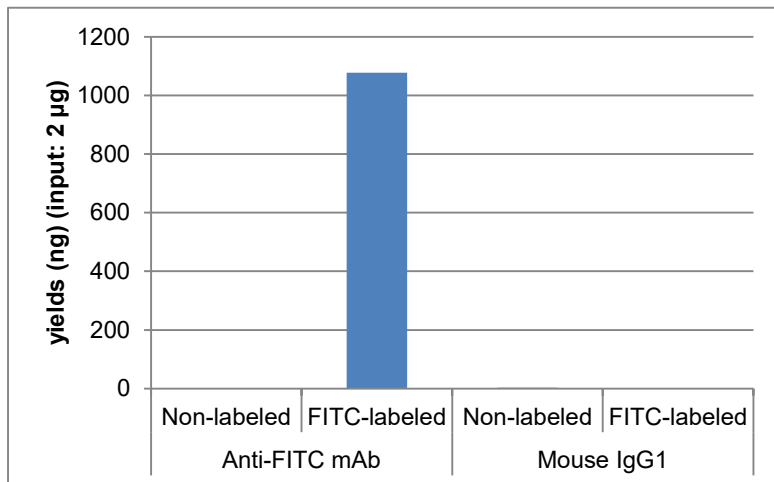
RNA Fluorescence *in situ* hybridization (RNA FISH)



Fluorescence in situ hybridization of MALAT1 ncRNA

Cells: HeLa
Probe: *MALAT1* ncRNA (RefSeq ID: NR_002819.3, region 6641-7113)
Antibody: Anti-FITC mAb (MBL, code no. M228-3), 1 μ g/mL

RNA immunoprecipitation

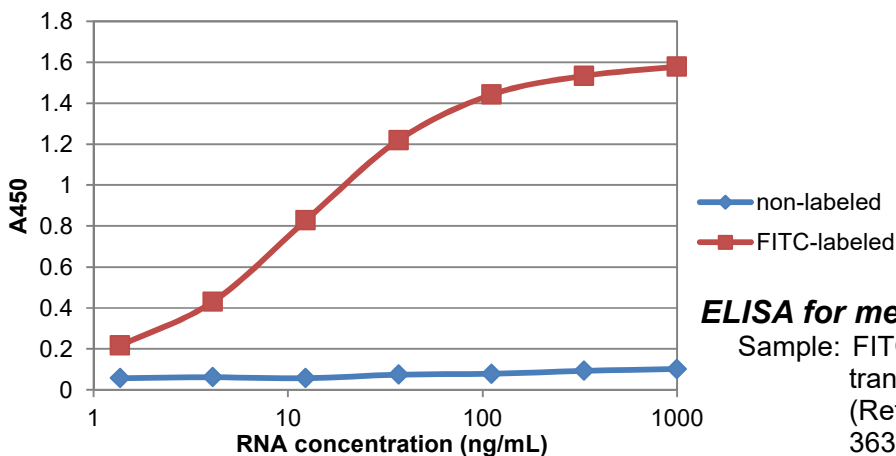


RNA immunoprecipitation of FITC-labeled RNA

Sample: FITC-labeled RNA synthesized by *in vitro* transcription from *lacZ*-encoding cDNA (RefSeq ID: NC_007779.1, region 363130-364149)
Antibody: Anti-FITC mAb (MBL, code no. M228-3), 10 μ g
Mouse IgG1 (isotype control) (MBL, code no. M075-3), 10 μ g

RNA ELISA

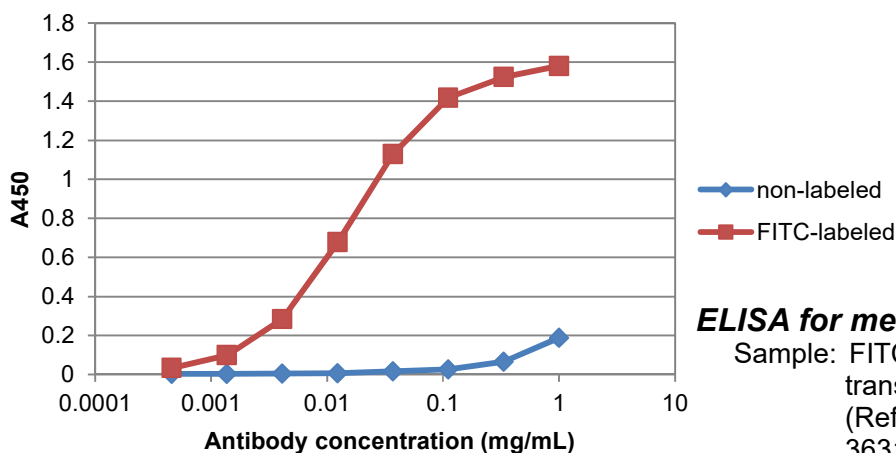
- 1) Add 50 μL /well of Poly-L-lysine solution (Sigma; code no. P4832-50ML) diluted with nuclease-free water to the 96-well plate. Incubate for 1 hr. at 37°C.
- 2) Wash the plate 3 times with nuclease-free water.
- 3) Sample preparation:
 - a) Prepare RNA samples by appropriate method (e.g., FITC labeling 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 FITC-labeled RNA

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

Antibody: Anti-FITC mAb (MBL, code no. M228-3), 1 $\mu\text{g}/\text{mL}$



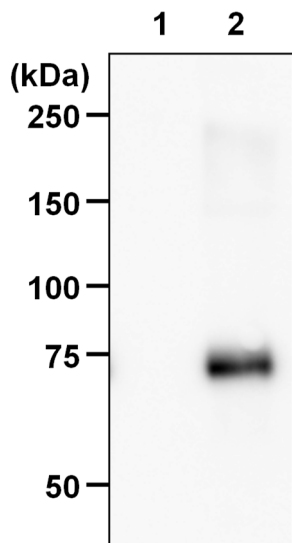
ELISA for measurement of FITC-labeled RNA

Sample: FITC-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-FITC mAb (MBL, code no. M228-3)

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% 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 the 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 90 sec. with ImageQuant LAS 4000 imaging system (Fujifilm). The condition for exposure and development may vary.



Western blotting analysis of FITC-conjugated protein

Lane 1: BSA, 100 pg

Lane 2: FITC-labeled BSA, 100 pg

Immunoblotted with Anti-FITC mAb (MBL, code no. M228-3)