

Anti-RFP mAb Cocktail

CODE No.	M208-3
CLONALITY	Monoclonal Cocktail
CLONE	1G9, 3G5 (mixed)
ISOTYPE	Mouse IgG2b κ , Mouse IgG1 κ
QUANTITY	50 μ L, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	RFP, recombinant protein
REACTIVITY	This clone reacts with RFP, DsRed, mCherry, mOrange and mPlumn. It does not cross-react with GFP.
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>Western blotting</u>	1 μ g/mL
<u>Immunoprecipitation</u>	5 μ g /sample
<u>Immunocytochemistry</u>	1 μ g/mL
<u>Flow cytometry</u>	0.1-1 μ g/mL

APPLICATION-REPORTED

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

REFERENCE 1) Fuse, A., *et al.*, *FEBS Lett.* **589**, 1430-1436 (2015) [WB]

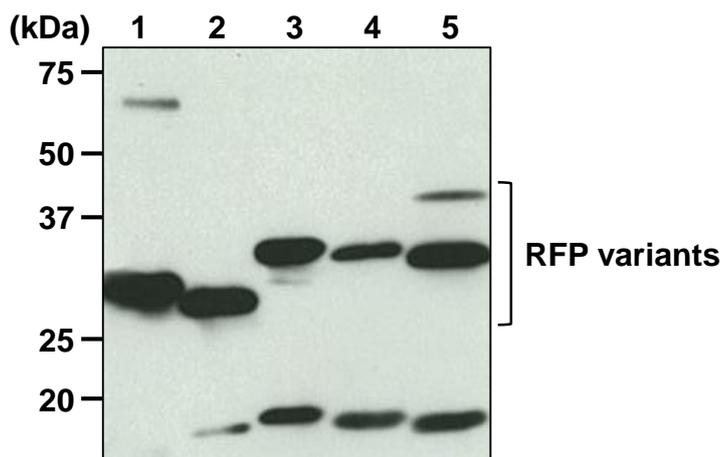
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For more information, please visit our web site <https://ruo.mbl.co.jp/>.

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

SDS-PAGE & Western blotting

- 1) Mix the sample with equal volume of Laemmili's sample buffer.
- 2) 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 (12.5% acrylamide) for electrophoresis.
- 3) 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.
- 4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
- 5) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
- 6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 7) Wash the membrane with PBS-T (5 min. x 3 times).
- 8) Incubate the membrane with 1:10,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 9) Wash the membrane with PBS-T (5 min. x 3 times).
- 10) Wipe excess buffer on the membrane, and 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 to an X-ray film in a dark room for 3 min. Develop the film as usual. The condition for exposure and development may vary.



Western blot analysis of RFP variants

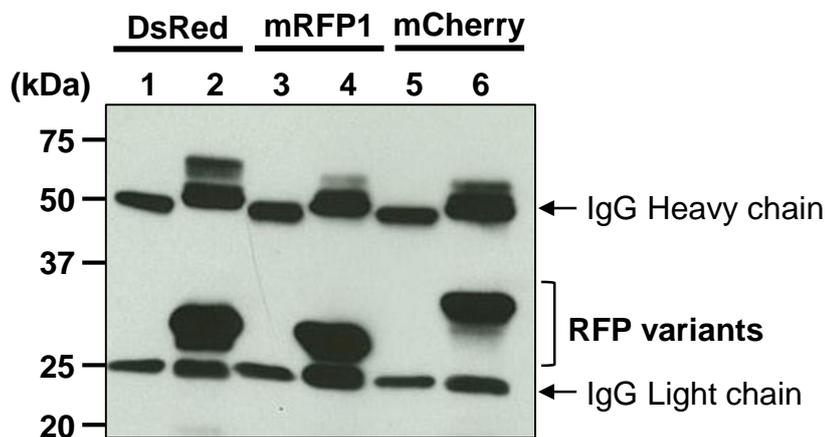
- Lane 1: DsRed
- Lane 2: mRFP1*
- Lane 3: mCherry*
- Lane 4: mOrange*
- Lane 5: mPlumn*

Immunoblotted with Anti-RFP mAb Cocktail (M208-3)

*Samples were provided by RIKEN.

Immunoprecipitation

- 1) Wash 2.5×10^6 cells 2 times with PBS and resuspend them with 1 mL of ice-cold Extraction buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% NP-40) containing appropriate protease inhibitors.
- 2) Incubate the cells on ice for 15 min., then sonicate briefly (up to 10 sec.).
- 3) Centrifuge the tube at $12,000 \times g$ for 5 min. at 4°C and transfer the supernatant to another tube.
- 4) Mix 20 μL of 50% protein A agarose beads slurry resuspended in 400 μL of IP buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40] with primary antibody as suggested in the **APPLICATIONS**. Incubate with gentle agitation for 30 min. at room temperature.
- 5) Wash the beads 1 time with 1 mL of IP buffer.
- 6) Add 400 μL of cell lysate, then incubate with gentle agitation for 1 hr. at room temperature.
- 7) Wash the beads 4 times with 1 mL of IP buffer.
- 8) Resuspend the beads in 20 μL of Laemmli's sample buffer, boil for 2 min. and centrifuge.
- 9) Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
- 10) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at $1 \text{ mA}/\text{cm}^2$ 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.
- 11) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C .
- 12) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) [5 min. x 3 times].
- 13) Incubate the membrane with 1 $\mu\text{g}/\text{mL}$ of Anti-RFP mAb Cocktail (MBL; code no. M208-3) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 14) Wash the membrane with PBS-T (5 min. x 3 times).
- 15) Incubate the membrane with 1:10,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 16) Wash the membrane with PBS-T (5 min. x 3 times).
- 17) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min.
- 18) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 19) Expose to an X-ray film in a dark room for 1 min. Develop the film as usual. The condition for exposure and development may vary.



Immunoprecipitation of RFP variants

- Lane 1 and 2: DsRed recombinant protein (5 μg)
- Lane 3 and 4: mRFP1/HEK293T cell lysate*
- Lane 5 and 6: mCherry recombinant protein* (5 μg)

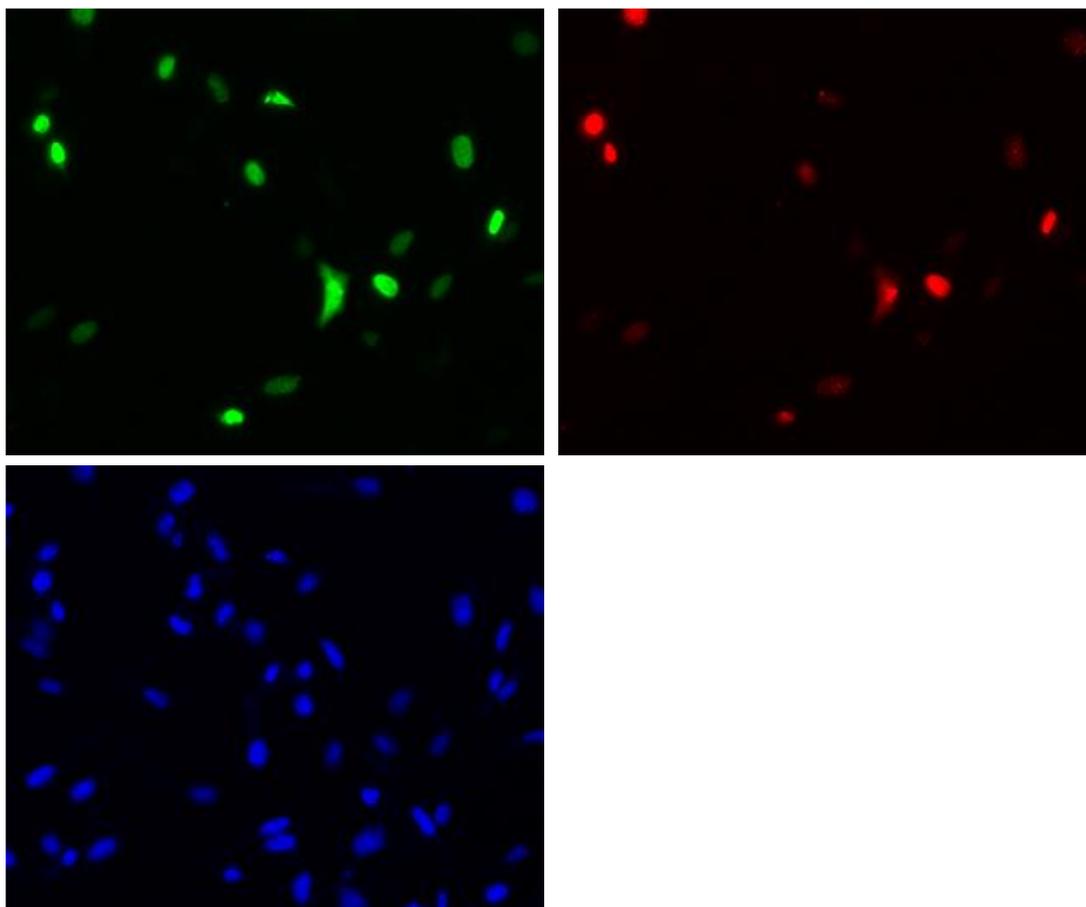
- Lane 1, 3 and 5: Mouse IgG1 (isotype control) (M075-3)
- Lane 2, 4 and 6: Anti-RFP mAb Cocktail (M208-3)

Immunoblotted with M208-3

*Samples were provided by RIKEN.

Immunocytochemistry

- 1) Spread the cells on a glass slide, then incubate in a CO₂ incubator for one night.
- 2) Remove the culture supernatant by careful aspiration.
- 3) Wash the slide 2 times with PBS.
- 4) Fix the cells with 4% paraformaldehyde (PFA)/PBS for 10 min. at room temperature (20~25°C).
- 5) Wash the slide 2 times with PBS.
- 6) Permeabilize the cells with 0.2% Triton X-100/PBS for 10 min. at room temperature.
- 7) Wash the slide 2 times with PBS.
- 8) Incubate the cells with the primary antibody diluted with PBS containing 2% fetal calf serum (FCS) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
- 9) Wash the slide 2 times with PBS.
- 10) Incubate the cells with 1:500 Alexa Fluor[®] 488 Goat Anti-mouse IgG (Invitrogen; code no. A11001) diluted with PBS containing 2% FCS for 30 min. at room temperature. Keep out light by aluminum foil.
- 11) Wash the slide 2 times with PBS.
- 12) Counterstain with DAPI for 5 min. at room temperature.
- 13) Wash the slide 2 times with PBS.
- 14) Promptly add mounting medium onto the slide, then put a cover slip on it.



Immunocytochemical detection of DsRed in HeLa transfectant

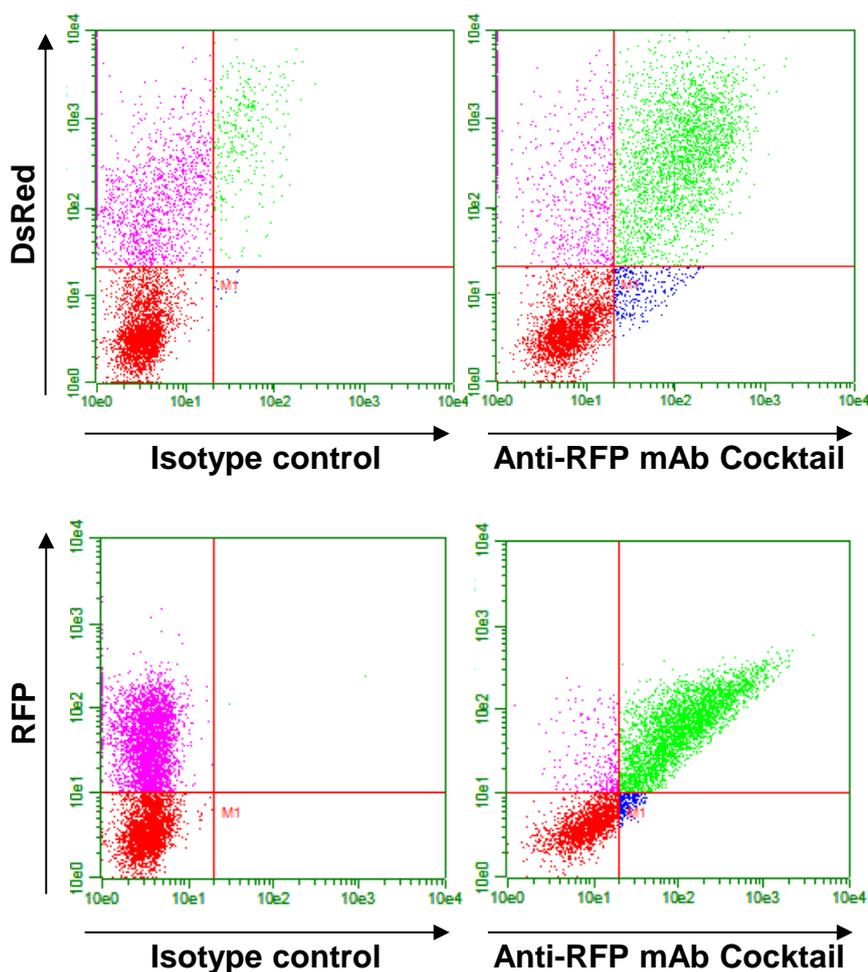
Green: Anti-RFP mAb Cocktail (M208-3)

Red: DsRed fluorescence

Blue: DAPI

Flow cytometric analysis

- 1) Wash 5×10^5 cells 3 times with 1 mL of washing buffer [PBS containing 2% fetal calf serum (FCS)].
- 2) Fix the cells with 4% paraformaldehyde (PFA)/PBS for 10 min. at room temperature (20~25°C).
- 3) Wash the cells 2 times with 1 mL of the washing buffer.
- 4) Permeabilize the cells with 0.2% Triton X-100/PBS for 10 min. at room temperature.
- 5) Wash the cells 2 times with 1 mL of washing buffer.
- 6) Add 40 μ L of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted in washing buffer. Mix well and incubate for 30 min. at 4°C.
- 7) Wash the cells 1 time with 1 mL of washing buffer.
- 8) Add 40 μ L of 1:500 Alexa Fluor® 488 Goat Anti-mouse IgG (Invitrogen; code no. A11001) diluted in washing buffer. Mix well and incubate for 30 min. at room temperature.
- 9) Wash the cells 1 time with 1 mL of washing buffer.
- 10) Resuspend the cells with 500 μ L of the washing buffer and analyze by a flow cytometer.



Flow cytometric analysis in HEK293T transfectant

Upper: DsRed fusion protein/HEK293T
Lower: mRFP1/HEK293T*

*Sample was provided by RIKEN.