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

MBL
A JSR Life Sciences Company

MONOCLONAL ANTIBODY

Anti-RFP mAb

Code No.	Clone	Subclass	Quantity	Concentration
M165-3	3G5	Mouse IgG1 κ	100 µL	1 mg/mL

BACKGROUND: Expression vector containing a tag sequence is commonly used to introduce and express a specific gene into a target cell. Red Fluorescent Protein (RFP) fusion protein expression system is preferably used in various laboratories, because it's easy monitoring of fusion proteins. This specific antibody for RFP is useful tool for monitoring of the fusion protein expression.

SOURCE: This antibody was purified from hybridoma (clone 3G5) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with C3H mouse lymphocyte immunized with RFP.

FORMULATION: 100 µg IgG in 100 µL volume of 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.

REACTIVITY: This antibody reacts with RFP fusion proteins on Immunoprecipitation, Immunocytochemistry and Flow cytometry.

APPLICATIONS:

Western blotting; Not tested*
*M204-3, M208-3 and PM005 are suitable for this application.
Immunoprecipitation; 5 µg/sample
Immunohistochemistry; Not tested
Immunocytochemistry; 1 µg/mL
Flow cytometry; 0.1-1 µg/mL

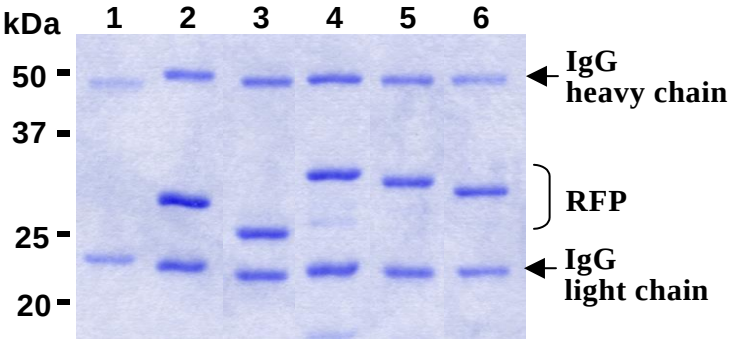
Detailed procedure is provided in the following **PROTOCOL**.

INTENDED USE:

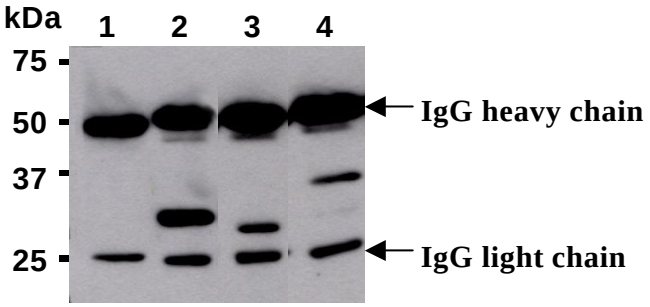
For Research Use Only. Not for use in diagnostic procedures.

REFERENCES:

1) Taguwa, S., *et al.*, *J. Virol.* **85**, 13185-13194 (2011) [IC]
2) Yamamoto, H., *et al., Mol. Biol. Cell* **21**, 2746-2755 (2010) [WB]



Immunoprecipitation of DsRed (1, 2), mRFP1* (3), mCherry* (4), mOrange* (5) and mPlum* (6) with isotype control (1) (MBL; code no. M075-8) or M165-8 (2-6). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and stained with CBB.*
**Sample number (3) to (6) are provided by RIKEN.*



Immunoprecipitation of DsRed fusion protein (1, 2), mRFP1* (3) and mCherry* (4) with mouse IgG1 (1) or M165-3 (2-4). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with anti-RFP monoclonal antibody (MBL; code no. M155-3).*
**Sample number (3) and (4) are provided by RIKEN.*

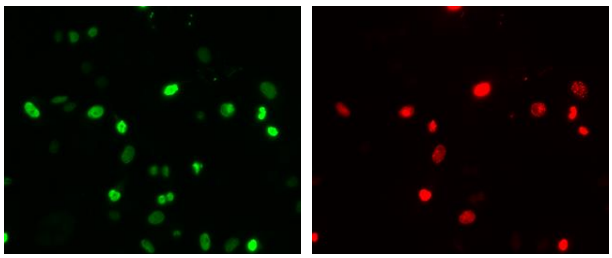
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOL:

Immunoprecipitation

1) Wash the transfectant cells 3 times with PBS and suspend with 10 volumes of cold Lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 15 minutes, then sonicate briefly (up to 10 seconds).

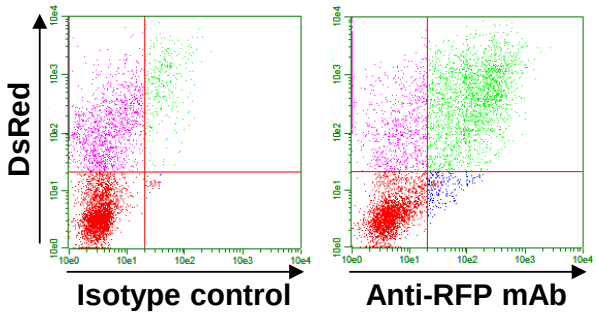
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
3) Add primary antibody as suggested in the **APPLICATIONS** into 200 µL of cell extract. Mix well and incubate with gentle agitation for 60-120 minutes at 4°C.
4) Add 20 µL of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
5) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
6) Resuspend the agarose in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
7) Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel for electrophoresis.
8) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). See the manufacture's manual for precise transfer procedure.
9) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
10) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
11) Incubate the membrane with 1 µg/mL of Anti-RFP mAb (MBL, code no. M155-3) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
12) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
13) Incubate the membrane with the 1:10,000 anti-IgG (Mouse) pAb-HRP (MBL, code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
14) Wash the membrane with PBS-T (5 minutes x 3).
15) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
16) Expose to an X-ray film in a dark room for 8 minutes. Develop the film as usual. The condition for exposure and development may vary.



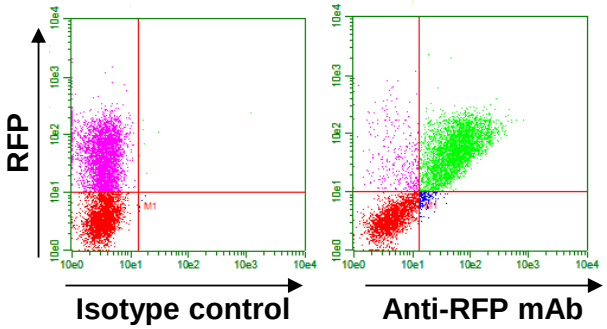
Immunocytochemical detection of DsRed fusion protein expressed in HeLa transfectants.
Left: Anti-RFP mAb (M165-3)
Right: DsRed fluorescence

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (For example, spread the 1 x 10^4 cells for one slide, then incubate in a CO_2 incubator overnight.)
2) Wash the cells 3 times with PBS.
3) Fix the cells with 4% paraformaldehyde (PFA)/PBS for 10 min. at room temperature (20~25°C).
4) Wash the slide twice with PBS.
5) Permeabilize the cells with 0.2% Triton X-100/PBS for 10 min. at room temperature.
6) Wash the slide twice with PBS.
7) Incubate the cells with the primary antibody diluted with PBS containing 2% fetal calf serum (FCS) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
8) Wash the slide twice with PBS.
9) 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 minutes at room temperature. Keep out light by aluminum foil.
10) Wash the slide twice with PBS.
11) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
12) Promptly add mounting medium onto the slide, then put a cover slip on it.



Flow cytometric analysis of DsRed (right) and isotypic control (left) on DsRed fusion protein expressed in HEK293T transfectants.
Fluorescence of DsRed is shown in the vertical axis.



Flow cytometric analysis of RFP (right) and isotypic control (left) on mRFP1 expressed in HEK293T transfectants.
Fluorescence of RFP is shown in the vertical axis.
Sample was provided by RIKEN.

Flow cytometric analysis

1) Wash the cells (5 x 10^5 cells/sample) twice with PBS.
2) Fix the cells with 4% paraformaldehyde (PFA)/PBS for 10 minutes at room temperature (20~25°C).
3) Wash the cells once with washing buffer (PBS/2% FCS).
4) Permeabilize the cells with 0.2% Triton X-100/PBS for 10 minutes at room temperature.
5) Wash the cells once with washing buffer.
6) Add 40 µL of the primary antibody at the concentration as suggested in the **APPLICATION** diluted in washing buffer. Mix well and incubate for 30 minutes at 4°C.
7) Wash the cells once with 1 mL of washing buffer.
8) Add 40 µL of 1:500 Alexa Fluor® 488 Goat Anti-Mouse IgG (Invitrogen, code no. A11001) diluted with washing buffer. Mix well and incubate for 30 min. at room temperature.
9) Wash the cells once with 1 mL of washing buffer.
10) Resuspend the cells with 500 µL of the Washing buffer and analyze by a flow cytometer.

RELATED PRODUCTS:

Please visit our website at https://ruo.mbl.co.jp/.