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MBL
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MONOCLONAL ANTIBODY

Anti-RFP mAb-Agarose

Code No.	Clone	Subclass	Quantity
M165-8	3G5	Mouse IgG1 κ	Gel: 200 µL

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: 400 µg of anti-RFP monoclonal antibody covalently coupled to 200 µL of agarose gel and provided as a 50% gel slurry suspended in PBS containing preservative (0.09% sodium azide) for a total volume of 400 µL.

*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at 4°C.

REACTIVITY: This antibody reacts with RFP fusion proteins on Immunoprecipitation.

APPLICATION:
Immunoprecipitation; 20 µL of gel slurry

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

INTENDED USE:
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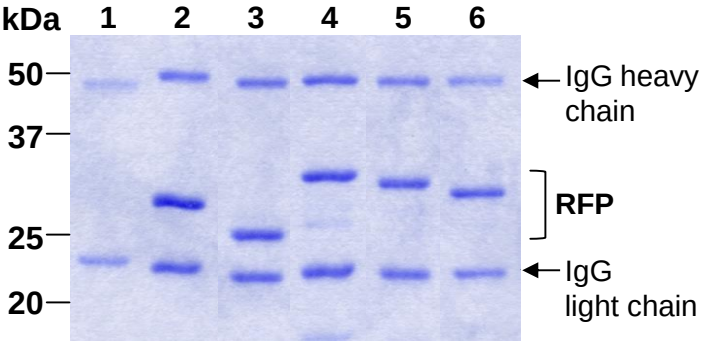
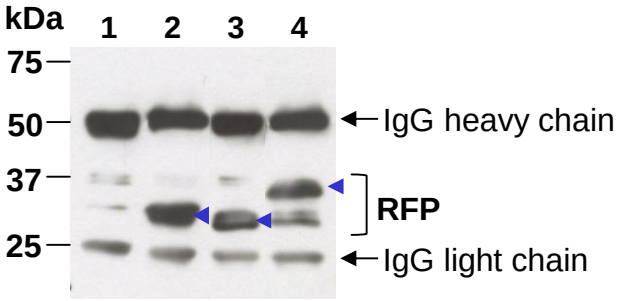
REFERENCES:
1) Södersten, E., *et al.*, *Nat commun*. **9**, 1226 (2018)
2) Hao, le T., *et al.*, *J. Neourosci*. **37**, 11559-11571 (2017)
3) Zhao, Q., *et al.*, *Sci. Rep*. **7**, 11250 (2017)
4) Kato, A., *et al.*, *J. Virol*. **85**, 9599-9613 (2011) [IP]
5) Yamamoto, H., *et al.*, *Mol. Biol. Cell* **121**, 2746-2755 (2010)

Clone 3G5 is used in these references.



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 (1,2), mRFP1* (3) and mCherry* (4) with isotype control (1) (MBL, code no. M075-8) or M165-8 (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) to (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 volume 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 **APPLICATION** into 200 µL of cell extract. Mix well and incubate with gentle agitation for 60-120 minutes at 4°C.
4) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
5) Resuspend the agarose with cold Lysis buffer.
6) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
7) Repeat steps 5) and 6) 2-4 times.
8) Resuspend the agarose in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
9) Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel for electrophoresis.
10) 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 manufacturer's manual for precise transfer procedure.
11) 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.
12) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
13) 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.)
14) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
15) Incubate the membrane with the 1:10,000 anti-IgG (H+L chain) (Mouse) pAb-HRP (MBL, code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
16) Wash the membrane with PBS-T (5 minutes x 3).
17) 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.
18) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary.

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