

MONOCLONAL ANTIBODY

# Anti-RFP mAb-Agarose

Code No.	Clone	Subclass	Quantity
M165-8	3G5	Mouse IgG1 $\kappa$	Gel: 200 $\mu$ L

**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  $\mu$ g of anti-RFP monoclonal antibody covalently coupled to 200  $\mu$ L 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  $\mu$ L.

\*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  $\mu$ L of gel slurry

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

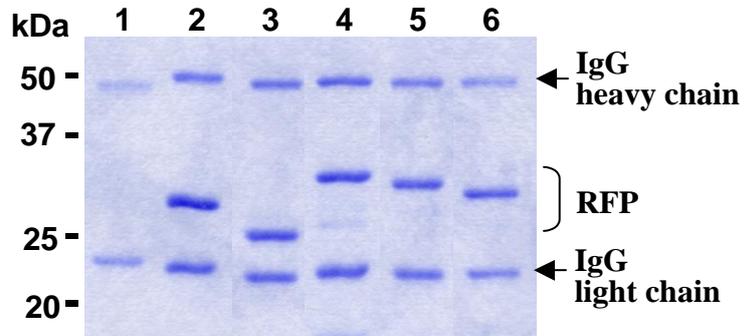
**INTENDED USE:**

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

**REFERENCES:**

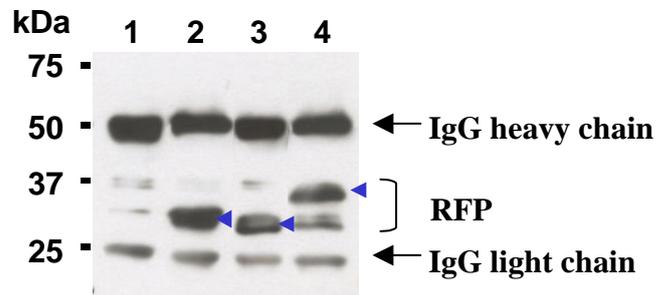
- 1) Södersten, E., *et al.*, *Nat commun.* **9**, 1226 (2018)
- 2) Hao, le T., *et al.*, *J. Neurosci.* **37**, 11559-11571 (2017)
- 3) Zhao, Q., *et al.*, *Sci. Rep.* **7**, 11250 (2017)
- 4) Kato, A., *et al.*, *J. Virol.* **85**, 9599-9613 (2011) [IP]
- 5) Yamamoto, H., *et al.*, *Mol. Biol. Cell*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



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\*Sample number (3) and (4) are provided by RIKEN.

**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 suggest in the **APPLICATION** into 200  $\mu$ L of cell extract. Mix well and incubate with gentle agitation for 60-120 minutes at 4°C.
- 4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
- 5) Resuspend the agarose in 20  $\mu$ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
- 6) Load 10  $\mu$ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel for electrophoresis.
- 7) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm<sup>2</sup> for 1 hour 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.
- 8) 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.
- 9) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 10) Incubate the membrane with 1  $\mu$ g/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 condition.)
- 11) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 12) Incubate the membrane with HRP-conjugated anti-mouse IgG antibody diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 13) Wash the membrane with PBS-T (5 minutes x 3 times).
- 14) 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.
- 15) 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.

## RELATED PRODUCTS:

### Antibodies

M048-3	Anti-GFP mAb (1E4)
D153-3	Anti-GFP mAb (RQ2)
D153-8	Anti-GFP mAb-Agarose (RQ2)
598	Anti-GFP pAb (polyclonal)
PM073	Anti-Renilla GFP pAb (polyclonal)
M208-3	Anti-RFP mAb Cocktail (1G9, 3G5)
M155-3	Anti-RFP mAb (8D6)
M165-3	Anti-RFP mAb (3G5)
M204-3	Anti-RFP mAb (1G9)
PM005	Anti-RFP pAb (polyclonal)
M180-3	Anti-HA-tag mAb (TANA2) (200 $\mu$ L)
561	Anti-HA-tag pAb (polyclonal) (0.1 mL)
561-8	Anti-HA-tag pAb-Agarose (polyclonal)
M132-3	Anti-HA-tag mAb (5D8)
M185-3L	Anti-DDDDK-tag mAb (FLA-1) (1 mL)
PM020	Anti-DDDDK-tag pAb (polyclonal)
PM020-8	Anti-DDDDK-tag pAb-Agarose (polyclonal)
M192-3	Anti-Myc-tag mAb (My3) (200 $\mu$ L)
M047-3	Anti-Myc-tag mAb (PL14)
M047-8	Anti-Myc-tag mAb-Agarose (PL14)

562	Anti-Myc-tag pAb (polyclonal) (0.1 mL)
D291-3	Anti-His-tag mAb (OGHis) (200 $\mu$ L)
D291-8	Anti-His-tag mAb-Agarose (OGHis)
M089-3	Anti-His-tag mAb (6C4)
M136-3	Anti-His-tag mAb (2D8)
PM032	Anti-His-tag pAb (polyclonal)
PM032-8	Anti-His-tag pAb-Agarose (polyclonal)
M167-3	Anti-V5-tag mAb (1H6)
M215-3	Anti-V5-tag mAb (OZA3)
PM003	Anti-V5-tag pAb (polyclonal)
PM003-8	Anti-V5-tag pAb-Agarose (polyclonal)
PM021	Anti-S-tag pAb (polyclonal)
PM070	Anti-E-tag pAb (polyclonal)
PM022	Anti-T7-tag pAb (polyclonal)
563	Anti-VSV-G-tag pAb (polyclonal)
M071-3	Anti-GST-tag mAb (3B2)
M209-3	Anti-GST-tag mAb (GT5)
PM022	Anti-GST-tag pAb (polyclonal)
M095-3	Anti-Luciferase mAb (2D4)
PM016	Anti-Luciferase pAb (polyclonal)
PM047	Anti-Renilla Luciferase pAb (polyclonal)
M094-3	Anti- $\beta$ -galactosidase mAb (5A3)
PM049	Anti- $\beta$ -galactosidase pAb (polyclonal)
M091-3	Anti-MBP (Maltose Binding Protein) mAb (1G12)
M013-3	Anti-Thioredoxin (Trx-tag) mAb (2C9)
PM015	Anti-CBD (Chitin Binding Domain) pAb (polyclonal)
PM071	Anti-Calmodulin Binding Protein-tag pAb (polyclonal)
M211-3	Anti-Strep-tag II mAb (4F1)
M214-3	Anti-mini-AID-tag mAb (1E4)

### Smart-IP series

3190	Magnetic Rack
M180-11	Anti-HA-tag mAb-Magnetic Beads (TANA2)
M132-11	Anti-HA-tag mAb-Magnetic Beads (5D8)
M185-11	Anti-DDDDK-tag mAb-Magnetic Beads (FLA-1)
M047-11	Anti-Myc-tag mAb-Magnetic Beads (PL14)
D291-11	Anti-His-tag mAb-Magnetic Beads (OGHis)
D153-11	Anti-GFP mAb-Magnetic Beads (RQ2)
M165-11	Anti-RFP mAb-Magnetic Beads (3G5)
M167-11	Anti-V5-tag mAb-Magnetic Beads (1H6)
M198-9	Anti-E-tag mAb-Magnetic beads (21D11)
D058-11	Anti-Multi Ubiquitin mAb-Magnetic Beads (FK2)
M075-11	Mouse IgG1 (isotype control)-Magnetic Beads
M076-11	Mouse IgG2a (isotype control)-Magnetic Beads
M077-11	Mouse IgG2b (isotype control)-Magnetic Beads
M081-11	Rat IgG2a (isotype control)-Magnetic Beads
M180-10	Anti-HA-tag mAb-Magnetic Agarose (TANA2)
M132-10	Anti-HA-tag mAb-Magnetic Agarose (5D8)
M185-10	Anti-DDDDK-tag mAb-Magnetic Agarose (FLA-1)
M047-10	Anti-Myc-tag mAb-Magnetic Agarose (PL14)
D291-10	Anti-His-tag mAb-Magnetic Agarose (OGHis)
D153-10	Anti-GFP mAb-Magnetic Agarose (RQ2)
M165-10	Anti-RFP mAb-Magnetic Agarose (3G5)
M167-10	Anti-V5-tag mAb-Magnetic Agarose (1H6)
M198-10	Anti-E-tag mAb-Magnetic Agarose (21D11)

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