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

MONOCLONAL ANTIBODY

Anti-GFP (Green Fluorescent Protein) mAb-Agarose

Code No.	Clone	Subclass	Quantity
D153-8	RQ2	Rat IgG2a	Gel: 200 µL

BACKGROUND: Since the detection of intracellular Aequorea Victria Green Fluorescent Protein (GFP) requires only irradiation by UV or blue light, it provides an excellent means for monitoring gene expression and protein localization in living cells. Agarose conjugated anti-GFP monoclonal antibody can detect GFP fusion protein on Immunoprecipitation.

SOURCE: This antibody was purified from hybridoma (clone RQ2) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell PAI with Wister rat lymphnode immunized with GFP purified from GFP expressed 293T cells by affinity chromatographic technique using mouse anti-GFP.

FORMULATION: 100 µg of anti-GFP monoclonal antibody covalently coupled to 200 µL of agarose gel and provided as a 50% gel slurry suspended in PBS containing preservative (0.1% ProClin 150) 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 GFP fusion protein on Immunoprecipitation. It reacts with EBFP, ECFP, EGFP, Venus and Sapphire.

APPLICATIONS:

Immunoprecipitation; 20 µL of gel slurry
2 µg of GFP can be precipitated with 20 µL of 50% gel slurry.

Chromatin immunoprecipitation; Not tested*
*It is reported that this antibody is used in Chromatin immunoprecipitation in the reference number 1) and 6).

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

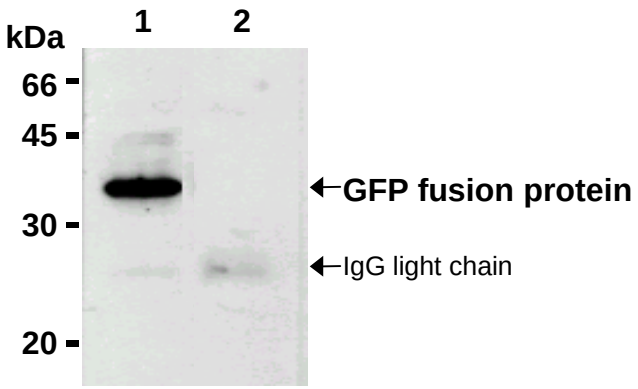
REFERENCES:

1) Celesnik, H., *et al., Biol. Open*. **2**, 424-431 (2013) [ChIP]
2) Cai, L., *et al., J. Biol. Chem*. **286**, 35915-35921 (2011) [IP]
3) Sato, Y., *et al., J. Biol. Chem*. **284**, 11873-11881 (2009) [IP]
4) Kato, A., *et al., J. Virol.* **82**, 6172-6189 (2008) [IP]
5) Dragone, L. L., *et al., PNAS*. **103**, 18202-18207 (2006) [IP]
6) Darzacq, X., *et al., J. Cell Biol.* **173**, 207-218 (2006) [ChIP]
7) Hayakawa, T., *et al., Plant Cell Physiol.* **47**, 891-904 (2006) [IP]
8) Obuse, C., *et al., Nat. Cell Biol*. **6**, 1135-1141 (2004) [IP]

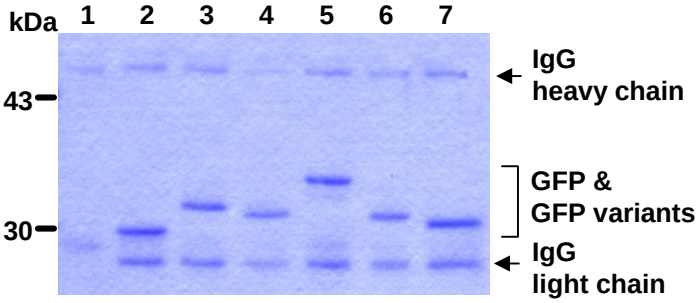
As this antibody is really famous all over the world, a lot of researches have been reported. These references are a part of such reports.

INTENDED USE:

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



Immunoprecipitation of GFP
Lane 1: IP with D153-8
Lane 2: IP with rat IgG2a-Agarose (code: M081-8)
Immunoblotted with M048-3



Immunoprecipitation of various fluorescent proteins
Lane 1, 2: GFP
Lane 3: EBFP
Lane 4: ECFP
Lane 5: EGFP
Lane 6: Venus
Lane 7: Sapphire
IP with isotype control (code: M081-8) (Lane 1)
IP with D153-8 (Lane 2-7)
Stained with CBB

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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 7.2), 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 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 the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C.
4) Wash the agarose 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 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
6) Load 10 µL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
7) 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% 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) Incubate the membrane with 1 µg/mL of Anti-GFP (Green Fluorescent Protein) mAb (clone 1E4, MBL; code no. M048-3) as primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
10) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 6 times).
11) 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 hour at room temperature.
12) Wash the membrane with PBS-T (5 minutes x 6 times).
13) 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.
14) Expose to an X-ray film in a dark room for 5 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)
D153-6	Anti-GFP mAb-Biotin (RQ2)
D153-A48	Anti-GFP mAb-Alexa Fluor® 488 (RQ2)
D153-A59	Anti-GFP mAb-Alexa Fluor® 594 (RQ2)
D153-A64	Anti-GFP mAb-Alexa Fluor® 647 (RQ2)
598	Anti-GFP pAb (polyclonal)
598-7	Anti-GFP pAb-HRP-DirecT (polyclonal)
PM073	Anti-Renilla GFP pAb (polyclonal)
M208-3	Anti-RFP mAb Cocktail (1G9, 3G5)
M155-3	Anti-RFP mAb (8D6)
M204-3	Anti-RFP mAb (1G9)
M165-3	Anti-RFP mAb (3G5)
M165-8	Anti-RFP mAb-Agarose (3G5)
PM005	Anti-RFP pAb (polyclonal)
M180-3	Anti-HA-tag mAb (TANA2) (200 µL)
M132-3	Anti-HA-tag mAb (5D8)
561	Anti-HA-tag pAb (polyclonal) (0.1 mL)
561-8	Anti-HA-tag pAb-Agarose (polyclonal)
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 µL)
M047-3	Anti-Myc-tag mAb (PL14)
M047-8	Anti-Myc-tag mAb-Agarose (PL14)
562	Anti-Myc-tag pAb (polyclonal) (0.1 mL)
M089-3	Anti-His-tag mAb (6C4)
M136-3	Anti-His-tag mAb (2D8)
D291-3	Anti-His-tag mAb (OGHis) (200 µL)
D291-8	Anti-His-tag mAb-Agarose (OGHis)
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)
PM021-8	Anti-S-tag pAb-Agarose (polyclonal)
PM070	Anti-E-tag pAb (polyclonal)
PM022	Anti-T7-tag pAb (polyclonal)
PM022-8	Anti-T7-tag pAb-Agarose (polyclonal)
563	Anti-VSV-G-tag pAb (polyclonal)
563-8	Anti-VSV-G-tag pAb-Agarose (polyclonal)
M071-3	Anti-GST-tag mAb (3B2)
M209-3	Anti-GST-tag mAb (GT5)
PM013	Anti-GST-tag pAb (polyclonal)

Smart-IP series

3190	Magnetic Rack
D153-11	Anti-GFP mAb-Magnetic Beads (RQ2)
M165-11	Anti-RFP mAb-Magnetic Beads (3G5)
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)
M215-11	Anti-V5-tag mAb-Magnetic Beads (OZA3)
M167-11	Anti-V5-tag mAb-Magnetic Beads (1H6)
M198-9	Anti-E-tag mAb-Magnetic beads (21D11)
D153-10	Anti-GFP mAb-Magnetic Agarose (RQ2)
M165-10	Anti-RFP mAb-Magnetic Agarose (3G5)
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)
M167-10	Anti-V5-tag mAb-Magnetic Agarose (1H6)
M198-10	Anti-E-tag mAb-Magnetic Agarose (21D11)

Other related antibodies and kits are also available.
Please visit our website at http://ruo.mbl.co.jp/