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Not for use in diagnostic procedures.

MBL
A JSR Life Sciences Company

MONOCLONAL ANTIBODY

Anti-GFP (Green Fluorescent Protein) mAb-Agarose

Code No.	Clone	Subclass	Quantity
D153-8	RQ2	Rat IgG2aκ	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.

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:

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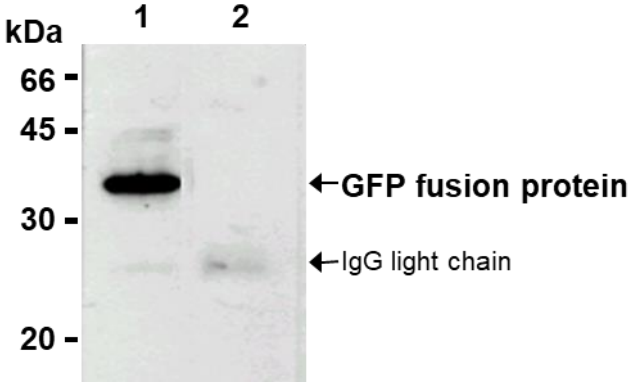
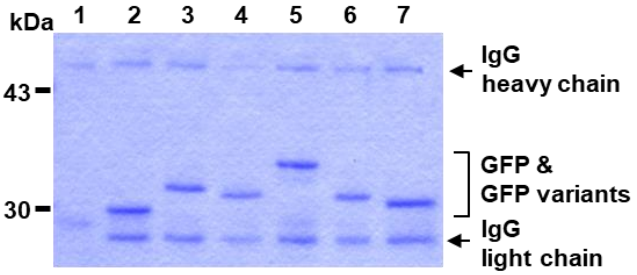


Immunoprecipitation of GFP
Lane 1: IP with Anti-GFP mAb-Agarose (MBL, code no. M153-8)
Lane 2: IP with Rat IgG2a (isotype control) -Agarose (MBL, code no. M081-8)
Immunoblotted with Anti-GFP mAb (MBL, code no. M048-3)



Immunoprecipitation of various fluorescent proteins
Lane 1, 2: GFP Lane 5: EGFP
Lane 3: EBFP Lane 6: Venus
Lane 4: ECFP Lane 7: Sapphire
IP with Rat IgG2a (isotype contrl)-Agarose (MBL, code no. M081-8) (Lane 1)
IP with Anti-GFP mAb-Agarose (MBL, code no. D153-8) (Lane 2-7)
Stained with CBB

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 7.2), 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) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant using a pipettor without disturbing the beads.
5) Resuspend the beads with cold Lysis buffer.
6) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant.
7) Repeat steps 5)-6) 3-5 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 sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out 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) 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 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
13) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 6 times).
14) 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.
15) Wash the membrane with PBS-T (5 minutes x 6 times).
16) 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.
17) 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.

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