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MONOCLONAL ANTIBODY

Anti-GFP (Green Fluorescent Protein) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D153-3	RQ2	Rat IgG2a κ	100 µL	1 mg/mL

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. Monoclonal anti-GFP 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 lymphonode immunized with GFP purified from GFP expressed 293T cells by affinity chromatographic technique using mouse anti-GFP.

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 GFP fusion protein on Immunoprecipitation and Immunocytochemistry. Clone RQ2 reacts with EBFP, ECFP, EGFP, Venus and Sapphire.

APPLICATIONS:

Immunoprecipitation; 0.5-2 µg/Sample
Western blotting; Not recommended*
Immunohistochemistry; Not recommended*
*MBL code no. M048-3 and 598 are suitable for these applications.
Immunocytochemistry; 2 µg/mL
Flow cytometry; Not tested

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

REFERENCES:

1) Zhou, H., *et al. Nat. Commun.* **7**, 10151 (2016) [WB, IP]
2) Luteijn, R. D., *et al., J. Immunol.* **193**, 1578-1589 (2014) [IP]
3) Sugiyama, T., *et al., Nucleic Acids Res.* **41**, 6674-6686 (2013) [IP]
4) Kunishima, S., *et al., Am. J. Hum. Genet.* **92**, 431-438 (2013) [IC]
5) Qi, Q., *et al., J. Biol. Chem.* **287**, 31482-31493 (2012) [Co-IP]
6) Sakurai, T., *et al., J. Cell Biol.* **183**, 339-352 (2008) [IP]
7) Obuse, C., *et al. Nat. Cell Biol.* **6**, 1135-1141 (2004) [IP]

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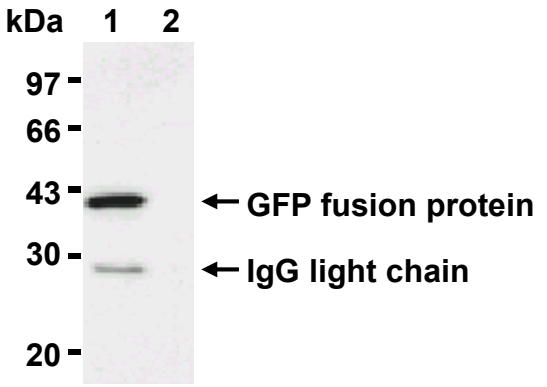
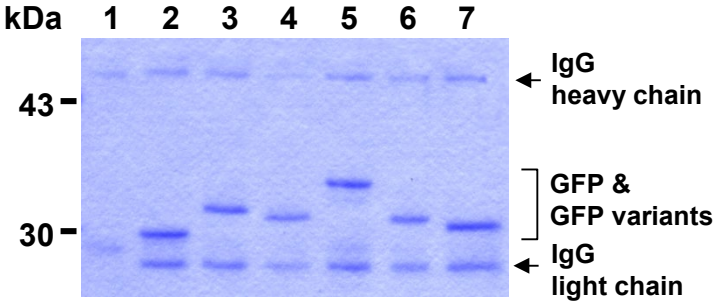


Immunoprecipitation of GFP fusion protein from transfectant with D153-3 (1) or isotype control (MBL; code no. M081-3) (2). *After immunoprecipitation with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with anti-GFP monoclonal antibody (MBL; code no. M048-3).*



Immunoprecipitation of GFP (1, 2), EBFP (3), ECFP (4), EGFP (5), Venus (6) and Sapphire (7) with isotype control (MBL; code no. M081-8) (1) or D153-8 (2-7). *After immunoprecipitation with the antibody, immunocomplex was resolved on SDS-PAGE and stained with CBB.*

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

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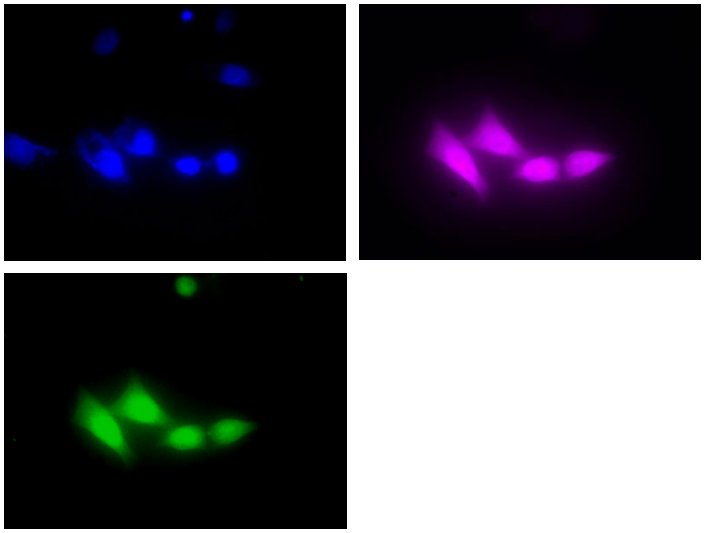
PROTOCOLS:

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 **APPLICATIONS** into 200 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20 µL of 50% protein G agarose resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 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 4)-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 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% MeOH). 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 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 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.

Immunocytochemistry

1) Spread the cells in the nutrient condition on a glass slide, then incubate in a CO_2 incubator for one night.
2) Remove the culture supernatant by careful aspiration.
3) Fix the cells by immersing the slide in 4% paraformaldehyde (PFA)/PBS for 10 minutes at room temperature (20~25°C).
4) Wash the slide 3 times with PBS.
5) Immerse the slide in 0.2% Triton X-100 in PBS for 10 minutes at room temperature.
6) Wash the slide with PBS.
7) Add 200 µL of the primary antibody diluted with 2% FCS/PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 30 minutes at room temperature.
8) Wash the slide 2 times with PBS.
9) Add 200 µL of 1:200 Alexa Fluor® 647 Goat Anti-Rat IgG (Invitrogen; code no. A21247) diluted with 2% FCS/PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
10) Wash the slide 2 times with PBS.
11) Counterstain with DAPI for 2 minutes at room temperature.
12) Wash the slide 2 times with PBS.
13) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
14) Promptly add mounting medium onto the slide, then put a cover slip on it.



Immunocytochemical detection of GFP expressed in HeLa with D153-3.
Blue: DAPI counterstain
Magenta: anti-GFP (D153-3)
Green: GFP own fluorescence

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