

MONOCLONAL ANTIBODY

Anti-GFP mAb-Agarose

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

BACKGROUND: Since the detection of intracellular Aequorea Victoria 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 Wistar rat lymphnode immunized with GFP purified from GFP expressed 293T cells by affinity chromatographic technique using mouse anti-GFP.

FORMULATION: 100 μ g of anti-GFP monoclonal antibody covalently coupled to 200 μ 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 μ 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 GFP fusion protein on Immunoprecipitation. It reacts with EBFP, ECFP, EGFP, Venus and Sapphire.

APPLICATION:

Immunoprecipitation; 20 μ L of gel slurry

2 μ g of GFP can be precipitated with 20 μ L of 50% gel slurry.

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

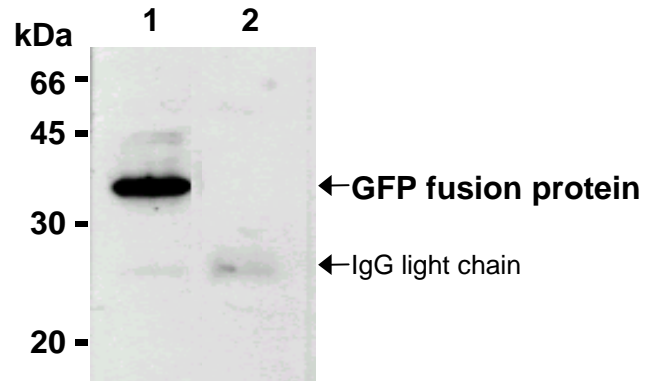
INTENDED USE:

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

REFERENCES:

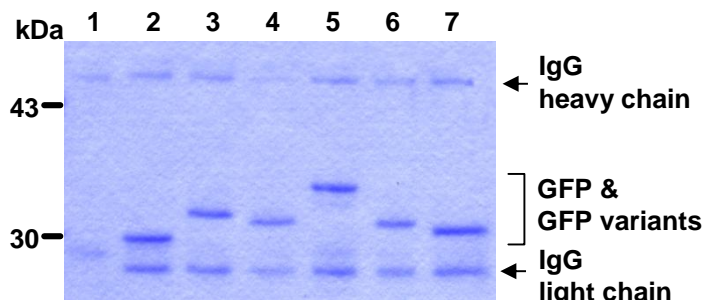
- 1) Cai, L., *et al.*, *J. Biol. Chem.* **286**, 35915-35921 (2011)
- 2) Sato, Y., *et al.*, *J. Biol. Chem.* **284**, 11873-11881 (2009)
- 3) Sakurai, T., *et al.*, *J. Cell Biol.* **183**, 339-352 (2008)
- 4) Kato, A., *et al.*, *J. Virol.* **82**, 6172-6189 (2008)
- 5) Dragone, L. L., *et al.* *PNAS.* **103**, 18202-18207 (2006)
- 6) Darzacq, X., *et al.* *J. Cell Biol.* **173**, 207-218 (2006)
- 7) Hayakawa, T., *et al.* *Plant Cell Physiol.* **47**, 891-904 (2006)
- 8) Obuse, C., *et al.* *Nat. Cell Biol.* **6**, 1135-1141 (2004)

Clone RQ2 is used in these references.



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 5: EGFP
Lane 3: EBFP Lane 6: Venus
Lane 4: ECFP Lane 7: Sapphire
IP with isotype control (code: M081-8) (Lane 1)
IP with D153-8 (Lane 2-7)
Stained with CBB

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/cm² 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 monoclonal antibody (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 the 1:10,000 HRP-conjugated anti-mouse IgG (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

- | | |
|----------|---|
| 598 | anti-GFP (polyclonal) |
| 598-7 | anti-GFP-HRP-Direct (polyclonal) |
| M048-3 | anti-GFP (1E4) |
| D153-3 | anti-GFP (RQ2) |
| D153-A48 | anti-GFP-Alexa Fluor [®] 488 (RQ2) |
| D153-A59 | anti-GFP-Alexa Fluor [®] 594 (RQ2) |
| D153-A64 | anti-GFP-Alexa Fluor [®] 647 (RQ2) |
| D153-8 | anti-GFP-Agarose (RQ2) |
| 561-8 | anti-HA-tag-agarose (polyclonal) |
| PM020-8 | anti-DDDDK-tag-agarose (polyclonal) |

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|---------|-------------------------------------|
| D291-8 | anti-His-tag-agarose (OGHis) |
| PM032-8 | anti-His-tag-agarose (polyclonal) |
| M165-8 | anti-RFP-agarose (3G5) |
| M047-8 | anti-Myc-tag-agarose (PL14) |
| PM021-8 | anti-S-tag-agarose (polyclonal) |
| PM022-8 | anti-T7-tag-agarose (polyclonal) |
| PM003-8 | anti-V5-tag-agarose (polyclonal) |
| 563-8 | anti-VSV-G-tag-agarose (polyclonal) |

Smart-IP series

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|--------|---|
| 3190 | Magnetic Rack |
| D153-9 | anti-GFP-Magnetic beads (RQ2) |
| M185-9 | anti-DDDDK-tag-Magnetic beads (FLA-1) |
| D291-9 | anti-His-tag-Magnetic beads (OGHis) |
| M165-9 | anti-RFP-Magnetic beads (3G5) |
| M132-9 | anti-HA-tag-Magnetic beads (5D8) |
| M180-9 | anti-HA-tag-Magnetic beads (TANA2) |
| M047-9 | anti-Myc-tag-Magnetic beads (PL14) |
| M167-9 | anti-V5-tag-Magnetic beads (1H6) |
| D058-9 | anti-Multi Ubiquitin-Magnetic beads (FK2) |

Protein Purification Kit

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|----------|--|
| 3305 | c-Myc-tagged Protein Mild Purification Kit |
| 3305A | c-Myc-tagged Protein Mild Purification Kit (Trial Kit) |
| 3306 | c-Myc-tagged Protein Mild Purification Gel with Elution Peptide (1 mL gel, 1 mg peptide) |
| 3307 | c-Myc-tagged Protein Mild Purification Gel with Elution Peptide (5 mL gel, 5 mg peptide) |
| 3300-205 | c-Myc-tag peptide (5 mg) |
| 3310 | His-tagged Protein Purification Kit |
| 3310A | His-tagged Protein Purification Kit (Trial Kit) |
| 3310-205 | His-tag peptide (10mg) |
| 3311 | His-tagged Protein Purification Gel with Elution Peptide (1 mL gel, 10 mg peptide) |
| 3312 | His-tagged Protein Purification Gel with Elution Peptide (5 mL gel, 50 mg peptide) |
| 3315 | V5-tagged Protein Purification Kit |
| 3315A | V5-tagged Protein Purification Kit (Trial Kit) |
| 3320 | HA-tagged Protein Purification Kit |
| 3320A | HA-tagged Protein Purification Kit (Trial Kit) |
| 3320-205 | HA-tag peptide (10 mg) |
| 3321 | HA-tagged Protein Purification Gel (1 mL) |
| 3325 | DDDDK-tagged Protein Purification Kit |
| 3325A | DDDDK-tagged Protein Purification Kit (Trial Kit) |
| 3325-205 | DDDDK-tag peptide (5 mg) |
| 3326 | DDDDK-tagged Protein Purification Gel with Elution Peptide (1 mL gel, 5 mg peptide) |
| 3327 | DDDDK-tagged Protein Purification Gel with Elution Peptide (5 mL gel, 25 mg peptide) |
| 3328 | DDDDK-tagged Protein Purification Gel (5 mL gel) |
| 3329 | DDDDK-tagged Protein Purification Gel (25 mL gel) |

Other related antibodies and kits are also available.

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