

MONOCLONAL ANTIBODY

Anti-GFP-Alexa Fluor® 647

Code No.	Clone	Subclass	Quantity	Concentration
D153-A64	RQ2	Rat IgG2a κ	50 μL	1 mg/mL

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. Monoclonal anti-GFP antibody can detect GFP fusion protein on Immunocytochemistry.

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

FORMULATION: 50 μg of IgG in 50 μL volume of PBS containing 1% BSA and 0.09% NaN₃.

*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 Immunocytochemistry. It reacts with EBFP, ECFP, EGFP, Venus and Sapphire.

APPLICATION:

Immunocytochemistry (for PFA fixed cells): 2-5 μg/mL

*This antibody is not suitable for alcohol fixation such as ethanol or methanol.

Please refer to the data sheet (MBL code no. D153-3) for other applications.

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

INTENDED USE:

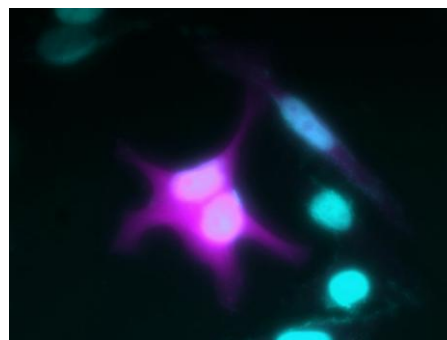
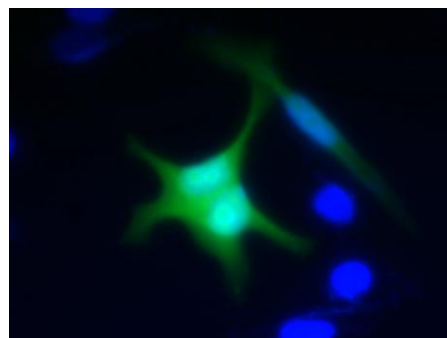
For Research Use Only. Not for use in diagnostic procedures.

REFERENCES:

- 1) Sato, Y., *et al.*, *J. Biol. Chem.* **284**, 11873-11881 (2009)
- 2) Sakurai, T., *et al.*, *J. Cell Biol.* **183**, 339-352 (2008)
- 3) Kato, A., *et al.*, *J. Virol.* **82**, 6172-6189 (2008)
- 4) Dragone, L. L., *et al.* *PNAS.* **103**, 18202-18207 (2006)
- 5) Darzacq, X., *et al.* *J. Cell Biol.* **173**, 207-218 (2006)

- 6) Hayakawa, T., *et al.* *Plant Cell Physiol.* **47**, 891-904 (2006)
- 7) Obuse, C., *et al.* *Nat. Cell Biol.* **6**, 1135-1141 (2004)

Clone RQ2 is used in these references.



Immunocytochemical detection of GFP expressed in HeLa using D153-A64.

Magenta: Alexa Fluor® 647
Green: GFP own fluorescence
Blue, Cyan: DAPI

PROTOCOL:

Immunocytochemistry

- 1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 1 x 10⁴ cells of transfectant cells for one slide, then incubate in a CO₂ incubator for one night.)
- 2) Wash the cells 3 times with PBS.
- 3) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde for 10 minutes at room temperature.
- 4) Wash the glass slide 3 times with PBS.
- 5) Immerse the slide in PBS containing 0.2% Triton X-100 for 10 minutes at room temperature.
- 6) Wash the glass slide 3 times with PBS.
- 7) Add Clear Back (MBL; code no. MTG-001) onto the cells and incubate for 10 minutes at room temperature.

- 8) Tip off Clear Back, add the primary antibody diluted with PBS containing 2% FCS as suggested in the **APPLICATION** onto the cells and incubate for 30 minutes at room temperature (Optimization of antibody concentration or incubation condition are recommended if necessary.)
- 9) Wash the glass slide 2 times with PBS.
- 10) Counter stain with DAPI for 2 minutes at room temperature.
- 11) Wash the glass slide 2 times with PBS.
- 12) Wipe excess buffer off the slide but take care not to touch the cells. Never leave the cells to dry.
- 13) Promptly add mounting medium onto the slide, then put a cover slip on it.

RELATED PRODUCTS:

Antibodies

598	anti-GFP (polyclonal)
598-7	anti-GFP HRP-DirecT (polyclonal)
M048-3	anti-GFP (1E4)
M048-A64	anti-GFP-Alexa Fluor [®] 647 (1E4)
D153-3	anti-GFP (RQ2)
D153-A48	anti-GFP-Alexa Fluor [®] 488 (RQ2)
D153-A59	anti-GFP-Alexa Fluor [®] 594 (RQ2)
D291-3	anti-His-tag (OGHis) (200 μ L)
D291-3S	anti-His-tag (OGHis) (50 μ L)
D291-7	anti-His-tag HRP-DirecT (OGHis)
D291-A48	anti-His-tag-Alexa Fluor [®] 488 (OGHis)
D291-A59	anti-His-tag-Alexa Fluor [®] 594 (OGHis)
D291-A64	anti-His-tag-Alexa Fluor [®] 647 (OGHis)
M089-3	anti-His-tag (6C4)
M136-3	anti-His-tag (2D8)
PM032	anti-His-tag (polyclonal)
PM032-8	anti-His-tag-agarose (polyclonal)
D153-8	anti-GFP-agarose (RQ2)
PM005	anti-RFP (polyclonal)
PM005-7	anti-RFP HRP-DirecT (polyclonal)
M155-3	anti-RFP (8D6)
M165-3	anti-RFP (3G5)
M165-8	anti-RFP-agarose (3G5)
M071-3	anti-GST-tag (3B2)
PM013	anti-GST-tag (polyclonal)
PM013-7	anti-GST-tag HRP-DirecT (polyclonal)
M013-3	anti-Thioredoxin (2C9)
M094-3	anti- β -galactosidase (5A3)
PM049	anti- β -galactosidase (polyclonal)
M095-3	anti-Luciferase (2D4)
PM016	anti-Luciferase (polyclonal)
PM047	anti-Renilla Luciferase (polyclonal)
PM020	anti-DDDDK-tag (polyclonal)
PM020-7	anti-DDDDK-tag HRP-DirecT (polyclonal)
PM020-8	anti-DDDDK-tag-agarose (polyclonal)
561	anti-HA-tag (polyclonal) (0.1 mL)
561-5	anti-HA-tag (polyclonal) (0.5 mL)
561-7	anti-HA-tag HRP-DirecT (polyclonal)
561-8	anti-HA-tag-agarose (polyclonal) (0.5 mL)

LABEL LICENSES:

This product is provided under an agreement between LIFE TECHNOLOGIES Corporation, and Medical & Biological Laboratories Co., LTD, and the manufacture, use, sale or import of this product may be subject to one or more U.S. patents, pending applications, and corresponding non-U.S. equivalents, owned by or licensed to LIFE TECHNOLOGIES Corporation. For information on purchasing a license to this product for any other use, contact LIFE TECHNOLOGIES Corporation, Molecular Probes Detection Technologies, Business Development, 29851 Willow Creek Road, Eugene, OR 97402, USA, Tel: (541) 465-8300. Fax: (541) 335-0504.

Alexa Fluor[®] is a registered trademark of Molecular Probes, Inc.

M132-3	anti-HA-tag (5D8)
M047-3	anti-Myc-tag (PL14)
M047-6	anti-Myc-tag-biotin (PL14)
M047-7	anti-Myc-tag HRP-DirecT (PL14)
M047-8	anti-Myc-tag-agarose (PL14)
M047-A48	anti-Myc-tag-Alexa Fluor [®] 488 (PL14)
M047-A59	anti-Myc-tag-Alexa Fluor [®] 594 (PL14)
M047-A64	anti-Myc-tag-Alexa Fluor [®] 647 (PL14)
562	anti-Myc-tag (polyclonal) (0.1 mL)
562-5	anti-Myc-tag (polyclonal) (0.5 mL)
PM021	anti-S-tag (polyclonal)
PM021-8	anti-S-tag-agarose (polyclonal)
PM022	anti-T7-tag (polyclonal)
PM022-8	anti-T7-tag-agarose (polyclonal)
M167-3	anti-V5-tag (1H6)
PM003	anti-V5-tag (polyclonal)
PM003-7	anti-V5-tag HRP-DirecT (polyclonal)
PM003-8	anti-V5-tag-agarose (polyclonal)
563	anti-VSV-G-tag (polyclonal)
563-8	anti-VSV-G-tag-agarose (polyclonal)

Smart-IP series

3190	Magnetic Rack
D291-3	anti-His-tag-magnetic beads (OGHis)
D153-9	anti-GFP-magnetic beads (RQ2)
M165-9	anti-RFP-magnetic beads (3G5)
M132-9	anti-HA-tag-magnetic beads (5D8)
M047-9	anti-Myc-tag-magnetic beads (PL14)
M167-9	anti-V5-tag-magnetic beads (1H6)

Isotype Controls

M081-3	Rat IgG2a isotype control (2H3)
M081-A64	Rat IgG2a isotype control-Alexa Fluor [®] 647 (2H3)

Protein Purification Kit

3310	His tagged Protein PURIFICATION KIT
3310A	His tagged Protein PURIFICATION KIT (Trial Kit)
3310-205	His tag peptide (10 mg)
3311	His tagged Protein PURIFICATION GEL (1 mL gel, 10 mg peptide)
3312	His tagged Protein PURIFICATION GEL (5 mL gel, 25 mg peptide)
3305	c-Myc tagged Protein MILD PURIFICATION KIT
3305A	c-Myc tagged Protein MILD PURIFICATION KIT (Trial Kit)
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)

Blocking Reagent

MTG-001	Clear Back
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Other related antibodies and kits are also available.
Please visit our website at <https://ruo.mbl.co.jp/>

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