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

MONOCLONAL ANTIBODY

Anti-GFP (Green Fluorescent Protein) mAb-Alexa Fluor™ 488

Code No.	Clone	Subclass	Quantity	Concentration
D153-A48	RQ2	Rat IgG2a κ	50 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 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 Wister 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% NaN_3.
*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:
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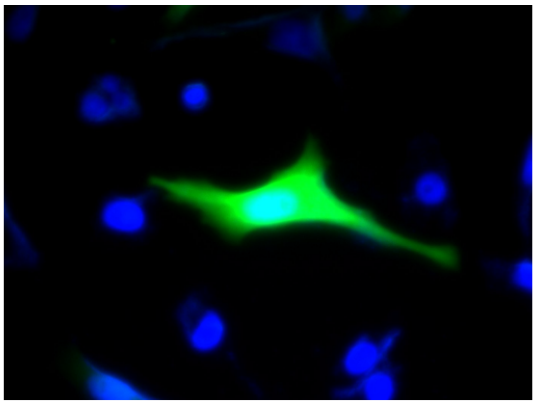
REFERENCES:
1) Kitamura, A., *et al., Genes Cells*. **22**, 521-534 (2017)
2) Sato, Y., *et al., J. Biol. Chem*. **284**, 11873-11881 (2009)
3) Sakurai, T., *et al., J. Cell Biol*. **183**, 339-352 (2008)
4) Kato, A., *et al., J. Virol*. **82**, 6172-6189 (2008)
5) Dragone, L. L., *et al. PNAS*. **103**, 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.



Immunocytochemical detection of GFP expressed in HeLa using D153-A48.
Green: Alexa Fluor® 488 and GFP own fluorescense
Blue: DAPI

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

PROTOCOL:

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (For example, spread 1 x 10^4 cells of transfectant cells for one slide, then incubate in a CO_2 incubator overnight.)
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 is recommended if necessary.)
9) Wash the glass slide twice with PBS.

10) Counter stain with DAPI for 2 minutes at room temperature.
11) Wash the glass slide twice 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.

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