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MONOCLONAL ANTIBODY

Anti- GFP (Green Fluorescent Protein) mAb

Code No.	Clone	Subclass	Quantity	Concentration
M048-3	1E4	Mouse IgG2b	100 µL	1 mg/mL

BACKGROUND: Green Fluorescent Protein (GFP) tagging provide an excellent means for monitoring gene expression and protein localization in living cells, so that it is widely accepted by molecular and cell biological research. Monoclonal anti-GFP antibody can detect GFP and its variants on Western blotting, Immunoprecipitation and Immunocytochemistry.

SOURCE: This antibody was purified from hybridoma (clone 1E4) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with recombinant full length GFP protein (246 aa).

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 on Western blotting, Immunoprecipitation and Immunocytochemistry. It reacts with EBFP, SEBFP, ECFP, SECFP, EGFP, SEGFP cpSEGFP, EYFP, Venus, cpVenus, R-pericam, and Sapphire.

APPLICATIONS:

Western blotting; 1 µg/mL
Immunoprecipitation; 5 µg/Sample
Immunohistochemistry; 10 µg/mL
Immunocytochemistry; 2 µg/mL
Flow cytometry; Not tested
Immunofluorescence; Not tested*

*It is reported that this antibody can be used in this application in the reference. Please visit our web site at https://ruo.mbl.co.jp/.

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

INTENDED USE:

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

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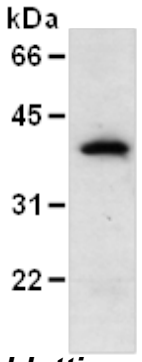
21) Nakamichi, I., *et al., Mol. Biol. Cell* **13**, 3441-3451 (2002) [WB]
22) Ozoe, F., *et al., Mol. Cell Biol.***22**, 7105-7119 (2002) [IP]
23) Ko, H. S., *et al., J. Biol. Chem.* **277**, 35386-35392 (2002) [IC]
24) Masuda, M., *et al., J. Biol. Chem.* **277**, 31014-31019 (2002) [WB]

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

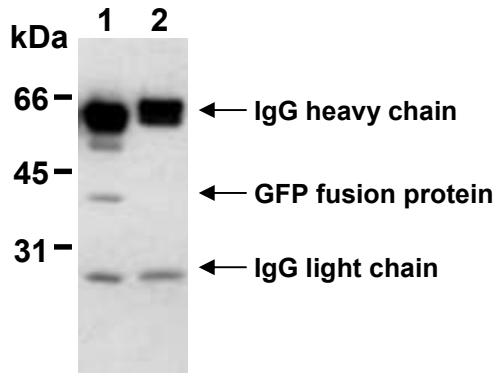
PROTOCOLS:



Western blotting analysis of GFP fused protein expression in transfectant using M048-3

SDS-PAGE & Western Blotting

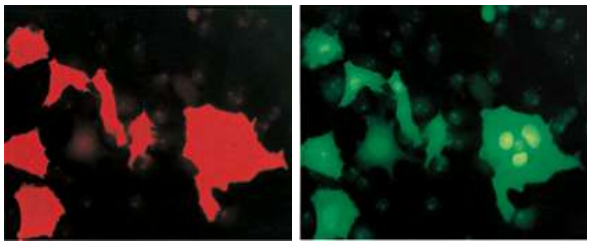
1) Boil the samples for 2 minutes and centrifuge. Load 10 µL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
2) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system. (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). See the manufacturer's manual for precise transfer procedure.
3) To reduce non-specific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
4) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature.
5) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 6).
6) Incubate the membrane with 1:10,000 of Anti-IgG (H+L chain) (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
7) Wash the membrane with PBS-T (5 minutes x 6).
8) Wipe excess buffer off the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute.
9) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
10) Expose the membrane onto an X-ray film in a dark room for 1 minute.
11) Develop the film under usual settings. The conditions for exposure and development may vary.



Immunoprecipitation of GFP from transfectant
Lane 1: IP with M048-3
Lane 2: IP with isotype control (M077-3)

Immunoprecipitation

1) Wash the 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 fresh tube.
3) Add the antibody at the amount as suggested in the **APPLICATIONS** to the supernatant containing approximately 300 µL total protein. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C.
4) Add 20 µL of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
5) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
6) Resuspend the beads with 1 mL of cold Lysis buffer.
7) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
8) Repeat steps 5)-7) 3-5 times.
9) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µL/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)



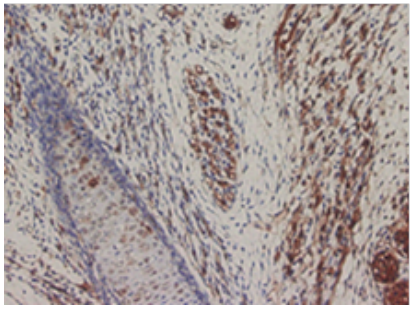
Immunocytochemical detection of GFP on 4% PFA fixed transfectant using M048-3 (left). Right panel is GFP own fluorescence.

Immunocytochemistry

Fixing:
1) Rinse the cells on glass coverslips in PBS, and immerse for 15 minutes in PBS containing 4% paraformaldehyde. Then rinse the coverslips 2 times for 5 minutes each in PBS.

Blocking:
1) Cover the cells with 3% H_2O_2 in PBS for 10 minutes at room temperature. Then rinse the coverslips 2 times for 5 minutes each in PBS.
2) Cover the cells with 10% normal goat serum (NGS) in PBS or Clear Back (human FcR blocking reagent, MBL code no. MTG-001) for 15 minutes to minimize non-specific adsorption of the antibodies to the cover slip (25-50 µL is usually sufficient).

Staining:
1) Remove the blocking buffer.
2) Incubate in primary antibody at the concentration suggested in **APPLICATIONS** diluted in 1% NGS in PBS for 1 hour at room temperature. (The concentration of antibody will depend on several variables and the abundance of the antigen.)
3) Wash the cells 3 times in PBS for 5 minutes each.
4) Incubate in Rhodamine-conjugated anti-mouse IgG antibody diluted in PBS for 1 hour at room temperature.
5) Wash the cells in PBS for 15 minutes.
6) Mounting and microscopic analysis.



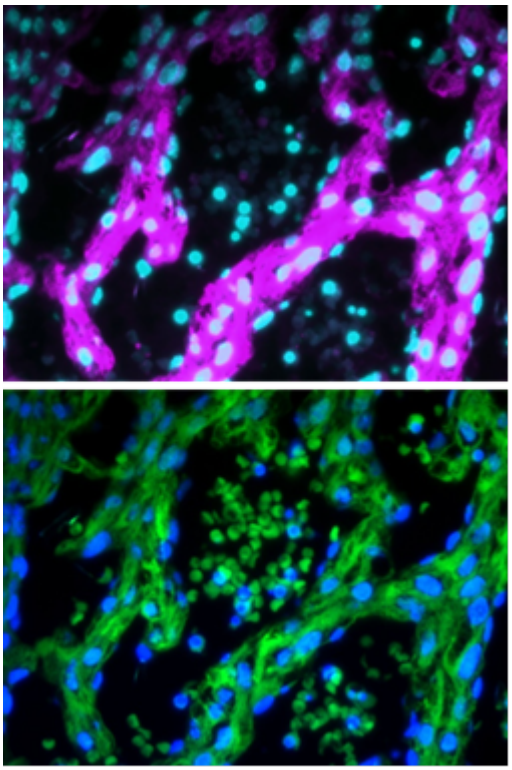
Immunohistochemical detection of GFP on paraffin embedded section of GFP transgenic mouse with M048-3.

Immunohistochemical staining for paraffin-embedded sections (DAB visualization)

1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Remove the slides from the PBS and cover each section with 3% H_2O_2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
5) Remove the slides from PBS, wipe gently around each section and cover tissues with blocking buffer (20 mM HEPES, 1% BSA, 135 mM NaCl) for 5 minutes to block non-specific staining. Do not wash.
6) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with blocking buffer as suggested in the **APPLICATIONS**.
7) Incubate the sections for 1 hour at room temperature.
8) Wash the slides 3 times in PBS for 5 minutes each.
9) Wipe gently around each section and cover tissues with Histostar™ (Ms + Rb) (MBL; code no. 8460). Incubate for 1 hour at room temperature. Wash as in step 8).
10) Visualize by reacting for 10 minutes with Histostar™ DAB Substrate Solution (MBL; code no. 8469). *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
11) Wash the slides in water for 5 minutes.
12) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
13) Now ready for mounting.

Immunohistochemical staining for paraffin-embedded sections (Visualization using Fluorochrome conjugated secondary antibody)

1) Refer to 1) – 3) of **Immunohistochemical staining for paraffin-embedded section (DAB visualization).**
2) Remove the slides from PBS, wipe gently around each section and cover tissues with blocking buffer (20 mM HEPES, 1% BSA, 135 mM NaCl) for 5 minutes to block non-specific staining. Do not wash.
3) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with blocking buffer as suggested in the **APPLICATIONS**.
4) Incubate the sections for 1 hour at room temperature.
5) Wash the slides 3 times in PBS for 5 minutes each.
6) Incubate in Alexa-Fluor® 647-conjugated anti-mouse IgG (Thermo Fisher Scientific; code no. A21237) diluted 1:200 in PBS for 1 hour at room temperature.
7) Wash the slides 3 times in PBS for 5 minutes each.
8) Counter stain with DAPI for 2 minutes at room temperature.
9) Wash the slides twice in PBS.
10) Now ready for mounting.



Immunohistochemical detection of GFP on paraffin embedded section of GFP transgenic mouse.
Magenta: M048-3
Green: GFP own fluorescence
Cyan, Blue: DAPI

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