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

MBL
A JSR Life Sciences Company

Anti-GFP (Green Fluorescent Protein) pAb

Code No.	Quantity	Form
598MS	20 µL	Purified IgG

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. Polyclonal anti-GFP antibody can detect GFP and its variants on Western blotting, Immunoprecipitation, Immunocytochemistry and Immunohistochemistry.

SOURCE: This antibody was purified from rabbit serum using ion exchange chromatography. The rabbit was immunized with recombinant GFP protein. The reactivity with recombinant partner and other *E. coli* components is absorbed using affinity chromatographic technique.

FORMULATION: 20 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody 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. This antibody also detects GFP-tagged proteins expressed in mammalian cell on Western blotting, Immunoprecipitation, Immunocytochemistry and Immunohistochemistry.

INTENDED USE:
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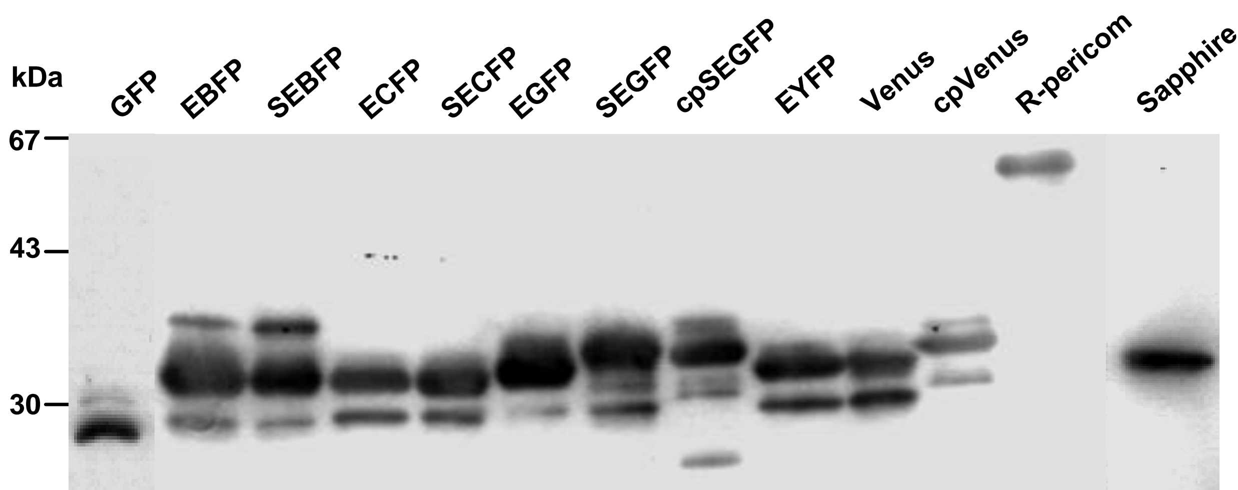
APPLICATIONS:
Western blotting; 1:1,000-1:5,000
Immunoprecipitation; 1 µL/Sample
Immunocytochemistry; 1:500
Immunohistochemistry; 1:1,000-1:2,000
Flow cytometry; Not tested
ChIP; Not tested*
Immuno-EM; Not tested *

*It is reported that this antibody can be used in ChIP[1)] and Immuno-EM[2)].

1) Fukazawa, J., *et al.*, *Plant J.* **62**, 1035-1045 (2010)
2) Nori, S., *et al.*, *PNAS.* **108**, 16825-16830 (2011)

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

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



Western blotting analysis of various fluorescent proteins

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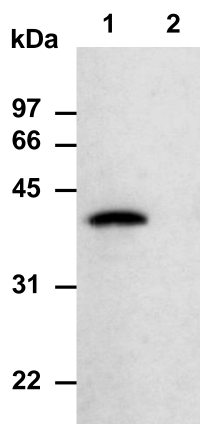
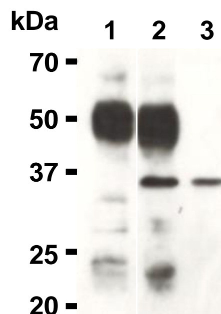


Western blotting analysis of GFP
Lane 1: GFP fusion protein transfectant
Lane 2: Parental cell
Immunoblotted with 598



Immunoprecipitation of GFP from HEK293T transfectant
Lane 1: Normal Rabbit IgG (PM035)
Lane 2: Anti-GFP pAb (598)
Lane 3: Input
Immunoblotted with Anti-GFP pAb (598)

PROTOCOLS:

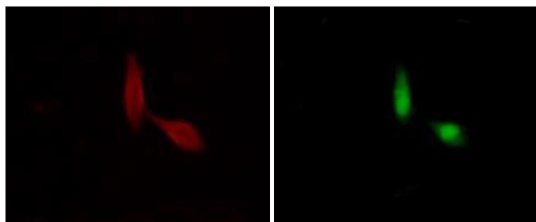
SDS-PAGE & Western blotting

1) Mix the sample with equal volume of Laemmli's sample buffer.
2) Boil all samples for 3~5 minutes and centrifuge. Load 10 µL of cell lysates or tissue homogenate (5~20 µg total protein) per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
3) 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.
4) 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.
5) 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. (The concentration of antibody will depend on the conditions.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
7) Incubate the membrane with the 1:10,000 Anti-IgG (Rabbit) pAb-HRP (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
8) Wash the membrane with PBS-T (5 minutes x 3).
9) Wipe excess buffer off the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute.
10) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
11) Expose to an X-ray film in a dark room for 3 minutes.
12) Develop the film as usual. The condition for exposure and development may vary.

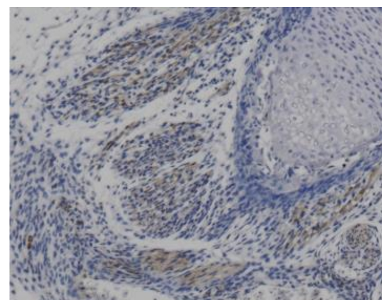
Immunoprecipitation

1) Wash 1 x 10^7 cells 3 times with PBS and suspend with 1 mL of cold NET-2 Buffer [500 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40], then sonicate briefly (up to 30 seconds). Incubate it on ice for 30 minutes.
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
3) Mix 20 µL of 50% protein A agarose beads slurry resuspended in PBS with 200 µL of NET-2 Buffer, then add Normal Rabbit IgG (MBL; code no. PM035) or Anti-GFP pAb (MBL; code no. 598) at the amount of suggested in the **APPLICATIONS** into each tube. Incubate with gentle agitation for 1 hour at room temperature.
4) Wash the beads once with 1 mL of NET-2 Buffer. Carefully discard the supernatant.
5) Add 200 µL of the supernatant (prepared from step 2) diluted 20-fold with NET-2 Buffer to the tube. Mix well and incubate with gentle agitation for 1 hour at room temperature.
6) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
7) Resuspend the agarose with NET-2 Buffer.
8) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
9) Repeat steps 7)-8) 3 times.
10) Resuspend the beads in 20 µL of Laemmli's sample buffer and centrifuge for 5 minutes. Boil for 5 minutes, Use 10 µL/lane for the SDS-PAGE analysis.

(See **SDS-PAGE & Western blotting**.)



Immunocytochemical detection of GFP expressed in transfectant
Left: Anti-GFP pAb (598)
Right: GFP own fluorescence



Immunohistochemical detection of GFP on E14.5 GFP mouse paraffin embedded section
Brown: Anti-GFP pAb (598)
Blue: Hematoxylin

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (For example, spread 2 x 10^4 cells per one well, then incubate in a CO_2 incubator overnight.)
2) Wash the slide twice with PBS.
3) Fix the cells by immersing the slide in 4% Paraformaldehyde Phosphate Buffer Solution (Wako; code no. 163-20145) for 10 minutes at room temperature.
4) Wash the slide twice with PBS.
5) Permeabilize the cells with PBS containing 0.2% Triton X-100 for 10 minutes at room temperature.
6) Wash the slide twice with PBS.
7) Incubate the cells with the primary antibody diluted with PBS as suggested in the **APPLICATIONS** for 1 hour at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
8) Wash the slide with twice with PBS.
9) Incubate the cells with 1:500 Goat anti-Rabbit IgG (H+L), Alexa Fluor® 594 (Thermo Fisher; code no. A11037) diluted with PBS for 30 minutes at room temperature. Keep out light by aluminum foil.
10) Counterstain with DAPI for 2 minutes at room temperature.
11) Wash the slide 3 times with PBS.
12) Wipe excess liquid off the slide but take care not to touch the cells. Never leave the cells to dry. Promptly add mounting medium onto the slide, then put a cover slip on it.

Immunohistochemical staining for paraffin-embedded sections

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 PBS and inactivate endogenous peroxidase with 3% H_2O_2 in PBS for 10 minutes.
5) Wash the slides with twice in PBS for 5 minutes.
6) Remove the slides from PBS, wipe gently around each section and cover tissues with blocking buffer [20 mM HEPES/1% BSA/135 mM NaCl (pH 7.4)] for 5 minutes at room temperature to block non-specific staining. Do not wash.
7) 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**. (The concentration of antibody will depend on the conditions.) 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 30 minutes at room temperature.
10) Wash the slides 3 times in PBS for 5 minutes each.
11) 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.
12) Wash the slides in water for 5 minutes.
13) 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.
14) Now ready for mounting.

REFERENCES:

Please visit our web site at https://ruo.mbl.co.jp.