

My select sampler set

Anti-GFP (Green Fluorescent Protein) pAb

Code No.	Quantity	Form
598MS	20 μ L	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 μ L 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:

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

APPLICATIONS:

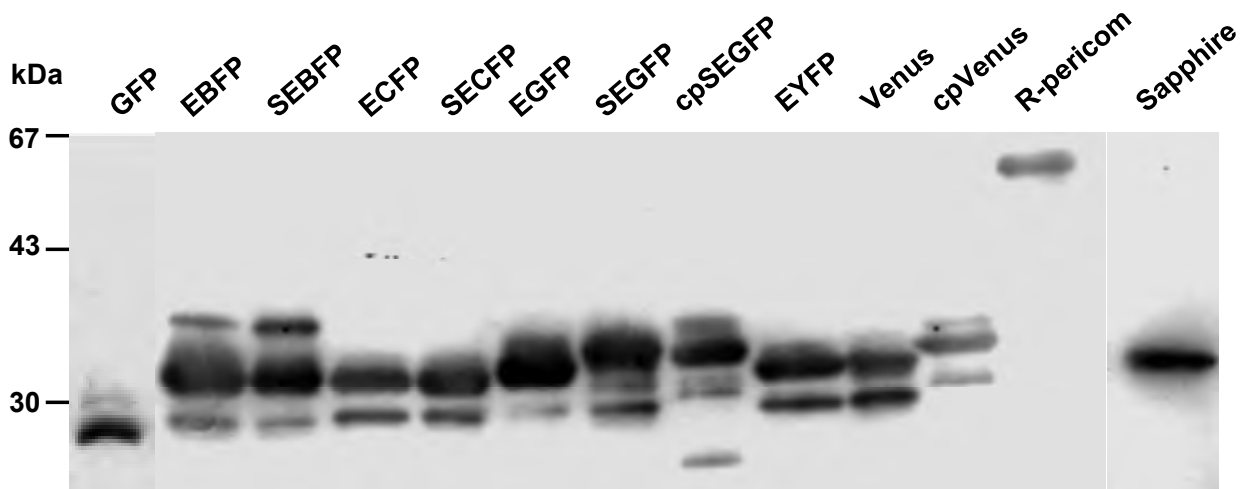
Western blotting; 1:1,000-1:5,000
Immunoprecipitation; 1 μ L/Sample
Immunocytochemistry; 1:500
Immunohistochemistry; 1:1,000-1:2,000
Flow cytometry; Not tested
ChIP; Not tested*
Immuno-EM; Not tested *

*It is reported that this antibody can be used in ChIP¹⁾ and Immuno-EM²⁾.

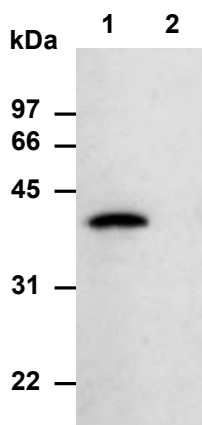
- 1) Fukazawa, J., *et al.*, *Plant J.* **62**, 1035-1045 (2010)
- 2) Nori, S., *et al.*, *PNAS.* **108**, 16825-16830 (2011)

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

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

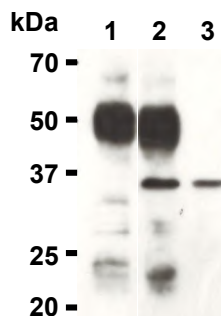


Western blotting analysis of various fluorescent proteins



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:

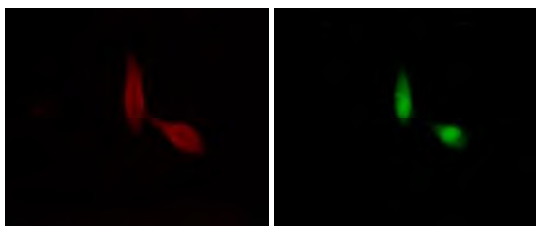
SDS-PAGE & Western blotting

- 1) Mix the sample with equal volume of Laemmli's sample buffer.
- 2) Boil all samples for 3~5 minutes and centrifuge. Load 10 μ L of cell lysates or tissue homogenate (5~20 μ g 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/cm² 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 10⁷ 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 μ L of 50% protein A agarose beads slurry resuspended in PBS with 200 μ L 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 μ L 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 μ L of Laemmli's sample buffer and centrifuge for 5 minutes. Boil for 5 minutes, Use 10 μ L/lane for the SDS-PAGE analysis.

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



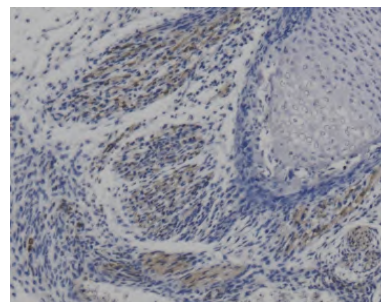
Immunocytochemical detection of GFP expressed in transfectant

Left: Anti-GFP pAb (598)

Right: GFP own fluorescence

Immunocytochemistry

- 1) Culture the cells in the appropriate condition on a glass slide. (For example, spread 2×10^4 cells per one well, then incubate in a CO₂ 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 detection of GFP on E14.5 GFP mouse paraffin embedded section

Brown: Anti-GFP pAb (598)

Blue: Hematoxylin

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% H₂O₂ 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:

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