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

POLYCLONAL ANTIBODY

Anti-Green Fluorescent Protein (GFP)

Code No.	Quantity	Form
598	100 µL	Purified IgG

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. 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 are absorbed using affinity chromatographic technique.

FORMULATION: This antibody solution contains with 0.3 M NaCl, 10 mM phosphate buffer, pH 8.0 and 0.09% sodium azide as preservative.

*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 is stable for one year from the date of purchase when stored at 4°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.

APPLICATIONS:

Western blotting; 1:1,000-1:5,000
for chemiluminescence detection system
Immunoprecipitation; 1 µL/Sample
Immunocytochemistry; 1:500
Immunohistochemistry; 1:1,000-1:2,000
Flow cytometry; Not tested
ChIP; Not tested (*reported in the reference 4))

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

INTENDED USE:

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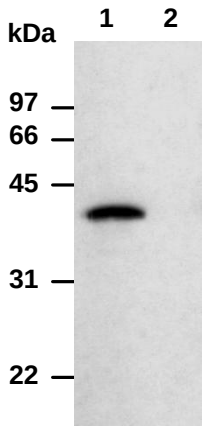
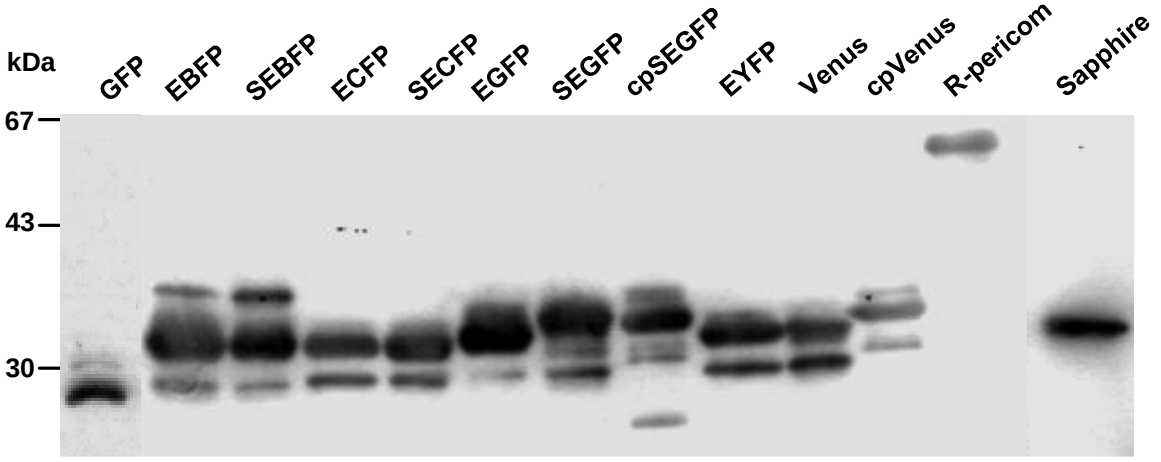


Western blot analysis of GFP
Lane 1: GFP fusion protein transfectant
Lane 2: Parental cell
Immunoblotted with 598



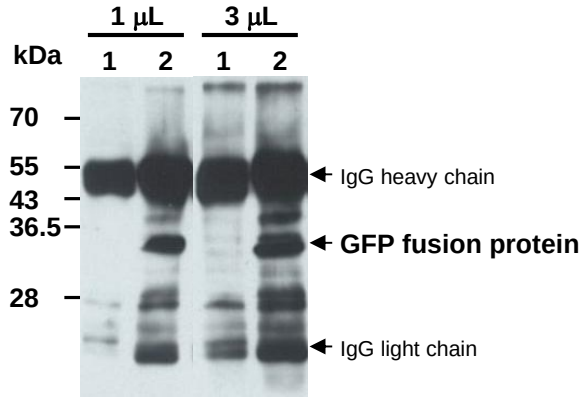
Western blot analysis of various fluorescent proteins

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL https://ruo.mbl.co.jp
e-mail support@mbl.co.jp, TEL 052-238-1904

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% MeOH). 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 PBS, pH 7.2 containing 1% skimmed milk 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 times).
7) Incubate the membrane with the 1:10,000 HRP-conjugated anti-rabbit IgG (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 6 times).
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 5 minutes.
12) Develop the film as usual. The condition for exposure and development may vary.



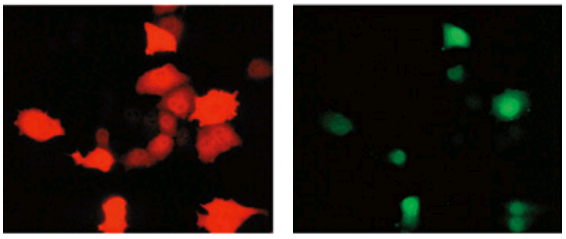
Immunoprecipitation of GFP

Lane 1: IP with Normal Rabbit IgG (code: PM035)
Lane 2: IP with 598
Immunoblotted with 598

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 tube.
3) Add the antibody at the amount of suggested in the **APPLICATIONS** to the supernatant containing approximately 100~500 µg total protein. Mix well and incubate with gentle agitation for 60-120 minutes at 4°C.
4) Add 20 µL of 50% protein A agarose beads resuspended in the Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
5) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
6) 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 expressed in transfectant

Left: anti-GFP (598)
Right: GFP own fluorescence
Photos were provided by Dr. Futoshi SHIBASAKI.
Tokyo Metropolitan Institute of Medical Science

Immunocytochemistry

Immunoperoxidase staining

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:

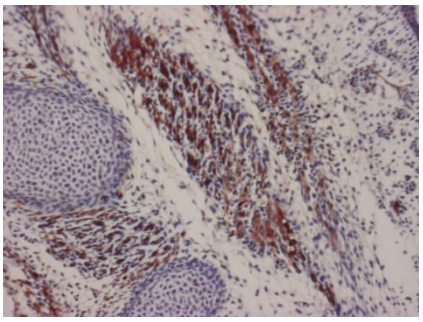
1) Cover the cells with 3% H_2O_2 in PBS for 10 minutes at room temperature. Then rinse the coverslips 2 times for 5 minutes each in PBS.
2) Remove the slides from PBS, wipe gently around each section and cover tissues with Protein Blocking Agent (Ultratech HRP Kit; MBL, code no. IM-2391) for 5 minutes to block non-specific staining. Do not wash.

Staining:

1) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggested in the **APPLICATIONS**.
2) Incubate the sections for 1 hour at room temperature.
3) Wash the slides 3 times in PBS for 5 minutes each.
4) Wipe gently around each section and cover tissues with Polyvalent Biotinylated Antibody (Ultratech HRP Kit).

Incubate for 15 minutes at room temperature. Wash as in step 3).

5) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase (Ultratech HRP Kit). Incubate for 15 minutes at room temperature. Wash as in step 3).
6) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 µL of 30% H_2O_2 in 150 mL PBS. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
7) Wash the slides in water for 5 minutes.
8) 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.
9) Now ready for mounting.



Immunohistochemical detection of GFP on GFP mouse paraffin embedded section with 598

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 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 Protein Blocking Agent (Ultratech HRP Kit; IMMUNOTECH, code no. IM-2391) 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 PBS containing 1% BSA 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 ENVISION+ Dual Link (Dako; code no. K4063). Incubate for 30 minutes at room temperature. Wash as in step 8).
10) Visualize by reacting for 10-20 minutes with DAB substrate kit (Dako; code no. K3466). *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.

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As this antibody is really famous all over the world, a lot of researches have been reported. These references are a part of such reports.

RELATED PRODUCTS

Antibodies

598	anti-GFP (polyclonal)
598-7	anti-GFP-HRP-DirecT (polyclonal)
M048-3	anti-GFP (1E4)
D153-3	anti-GFP (RQ2)
D153-A48	anti-GFP-Alexa Fluor® 488 (RQ2)
D153-A59	anti-GFP-Alexa Fluor® 594 (RQ2)
D153-A64	anti-GFP-Alexa Fluor® 647 (RQ2)
D153-8	anti-GFP-Agarose (RQ2)
M192-3	anti-Myc-tag (My3) (200 µL)
M192-3S	anti-Myc-tag (My3) (50 µL)
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)
M180-3	anti-HA-tag (TANA2) (200 µL)
M180-3S	anti-HA-tag (TANA2) (50 µL)
M180-7	anti-HA-tag-HRP-DirecT (TANA2)
M180-A48	anti-HA-tag-Alexa Fluor® 488 (TANA2)
M180-A59	anti-HA-tag-Alexa Fluor® 594 (TANA2)
M180-A64	anti-HA-tag-Alexa Fluor® 647 (TANA2)
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)
M132-3	anti-HA-tag (5D8)
M185-3L	anti-DDDDK-tag (FLA-1) (1 mL)
M185-3LL	anti-DDDDK-tag (FLA-1) (5 mL)
M185-3S	anti-DDDDK-tag (FLA-1) (50 µL)
M185-A48	anti-DDDDK-tag-Alexa Fluor® 488 (FLA-1)
M185-A59	anti-DDDDK-tag-Alexa Fluor® 594 (FLA-1)
M185-A64	anti-DDDDK-tag-Alexa Fluor® 647 (FLA-1)
PM020	anti-DDDDK-tag (polyclonal)
PM020-7	anti-DDDDK-tag-HRP-DirecT (polyclonal)
PM020-8	anti-DDDDK-tag-Agarose (polyclonal)
D291-3	anti-His-tag (OGHis) (200 µL)
D291-3S	anti-His-tag (OGHis) (50 µL)
D291-6	anti-His-tag-Biotin (OGHis)
D291-7	anti-His-tag-HRP-DirecT (OGHis)
D291-8	anti-His-tag-Agarose (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)
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)
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)

Smart-IP series

3190	Magnetic Rack
M047-9	anti-Myc-tag-Magnetic beads (PL14)
M180-9	anti-HA-tag-Magnetic beads (TANA2)
M132-9	anti-HA-tag-Magnetic beads (5D8)
M185-9	anti-DDDDK-tag-Magnetic beads (FLA-1)
D291-9	anti-His-tag-Magnetic beads (OGHis)
D153-9	anti-GFP-Magnetic beads (RQ2)
M165-9	anti-RFP-Magnetic beads (3G5)
M167-9	anti-V5-tag-Magnetic beads (1H6)

Protein Purification Kit

3305	c-Myc-tagged Protein MILD PURIFICATION KIT
3305A	c-Myc-tagged Protein MILD PURIFICATION KIT (Trial Kit)
3306	c-Myc-tagged Protein MILD PURIFICATION GEL (1 mL gel, 1 mg peptide)
3307	c-Myc-tagged Protein MILD PURIFICATION GEL (5 mL gel, 5 mg peptide)
3300-205	c-Myc tag peptide (5 mg)
3310	His-tagged Protein PURIFICATION KIT
3310A	His-tagged Protein PURIFICATION KIT (Trial Kit)
3310-205	His-tag peptide (10mg)
3311	His-tagged Protein PURIFICATION GEL (1 mL gel, 10 mg peptide)
3312	His-tagged Protein PURIFICATION GEL (5 mL gel, 50 mg peptide)
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)
3321	HA-tagged Protein PURIFICATION GEL
3320-205	HA-tag peptide (10mg)
3325	DDDDK-tagged Protein PURIFICATION KIT
3325A	DDDDK-tagged Protein PURIFICATION KIT (Trial Kit)
3325-205	DDDDK-tag peptide (5 mg)
3326	DDDDK-tagged Protein PURIFICATION GEL (1 mL gel, 5 mg peptide)
3327	DDDDK-tagged Protein PURIFICATION GEL (5 mL gel, 25 mg peptide)
3328	DDDDK-tagged Protein PURIFICATION GEL (5 mL gel)
3329	DDDDK-tagged Protein PURIFICATION GEL (25 mL gel)

Other related antibodies and kits are also available.
Please visit our website at https://ruo.mbl.co.jp/