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

Anti-Renilla GFP pAb

CODE No.	PM073
CLONALITY	Polyclonal
ISOTYPE	Rabbit Ig, affinity purified
QUANTITY	100 μL
SOURCE	Purified Ig from rabbit serum
IMMUNOGEN	*Renilla reniformis* GFP, recombinant protein
FORMURATION	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.

APPLICATIONS-CONFIRMED

Western blotting	1:1,000 for chemiluminescence detection system
Immunoprecipitation	1 μL/sample
Immunocytochemistry	1:2,000
Flow cytometory	1:2,000

For more information, please visit our web site **http://ruo.mbl.co.jp/**

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
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RELATED PRODUCTS

Antibodies

PM073	Anti-Renilla GFP pAb
598	Anti-GFP pAb (polyclonal)
598-7	Anti-GFP pAb-HRP-DirecT (polyclonal)
M048-3	Anti-GFP mAb (1E4)
D153-3	Anti-GFP mAb (RQ2)
D153-A48	Anti-GFP mAb-Alexa Fluor® 488 (RQ2)
D153-A59	Anti-GFP mAb-Alexa Fluor® 594 (RQ2)
D153-A64	Anti-GFP mAb-Alexa Fluor® 647 (RQ2)
D153-8	Anti-GFP mAb-Agarose (RQ2)
PM005	Anti-RFP pAb (polyclonal)
PM005-7	Anti-RFP pAb-HRP-DirecT (polyclonal)
M155-3	Anti-RFP mAb (8D6)
M165-3	Anti-RFP mAb (3G5)
M165-8	Anti-RFP mAb-Agarose (3G5)
M192-3	Anti-Myc-tag mAb (My3) (200 µL)
M047-3	Anti-Myc-tag mAb (PL14)
M047-6	Anti-Myc-tag mAb-Biotin (PL14)
M047-7	Anti-Myc-tag mAb-HRP-DirecT (PL14)
M047-8	Anti-Myc-tag mAb-Agarose (PL14)
M047-A48	Anti-Myc-tag mAb-Alexa Fluor® 488 (PL14)
M047-A59	Anti-Myc-tag mAb-Alexa Fluor® 594 (PL14)
M047-A64	Anti-Myc-tag mAb-Alexa Fluor® 647 (PL14)
562	Anti-Myc-tag pAb (polyclonal) (0.1 mL)
562-5	Anti-Myc-tag pAb (polyclonal) (0.5 mL)
M180-3	Anti-HA-tag mAb (TANA2) (200 µL)
M180-7	Anti-HA-tag mAb-HRP-DirecT (TANA2)
M180-A48	Anti-HA-tag mAb-Alexa Fluor® 488 (TANA2)
M180-A59	Anti-HA-tag mAb-Alexa Fluor® 594 (TANA2)
M180-A64	Anti-HA-tag mAb-Alexa Fluor® 647 (TANA2)
561	Anti-HA-tag pAb (polyclonal) (0.1 mL)
561-5	Anti-HA-tag pAb (polyclonal) (0.5 mL)
561-7	Anti-HA-tag pAb-HRP-DirecT (polyclonal)
561-8	Anti-HA-tag pAb-Agarose (polyclonal)
M132-3	Anti-HA-tag mAb (5D8)
M185-3L	Anti-DDDDK-tag mAb (FLA-1) (1 mL)
M185-3LL	Anti-DDDDK-tag mAb (FLA-1) (5 mL)
M185-A48	Anti-DDDDK-tag mAb-Alexa Fluor® 488 (FLA-1)
M185-A59	Anti-DDDDK-tag mAb-Alexa Fluor® 594 (FLA-1)
M185-A64	Anti-DDDDK-tag mAb-Alexa Fluor® 647 (FLA-1)
PM020	Anti-DDDDK-tag pAb (polyclonal)
PM020-7	Anti-DDDDK-tag pAb-HRP-DirecT (polyclonal)
PM020-8	Anti-DDDDK-tag pAb-Agarose (polyclonal)
D291-3	Anti-His-tag mAb (OGHis) (200 µL)
D291-6	Anti-His-tag mAb-Biotin (OGHis)
D291-7	Anti-His-tag mAb-HRP-DirecT (OGHis)
D291-8	Anti-His-tag mAb-Agarose (OGHis)
D291-A48	Anti-His-tag mAb-Alexa Fluor® 488 (OGHis)
D291-A59	Anti-His-tag mAb-Alexa Fluor® 594 (OGHis)
D291-A64	Anti-His-tag mAb-Alexa Fluor® 647 (OGHis)
M089-3	Anti-His-tag mAb (6C4)
M136-3	Anti-His-tag mAb (2D8)
PM032	Anti-His-tag pAb (polyclonal)
PM032-8	Anti-His-tag pAb-Agarose (polyclonal)
M167-3	Anti-V5-tag mAb (1H6)
PM003	Anti-V5-tag pAb (polyclonal)
PM003-7	Anti-V5-tag pAb-HRP-DirecT (polyclonal)
PM003-8	Anti-V5-tag pAb-Agarose (polyclonal)
PM070	Anti-E-tag pAb (polyclonal)
PM071	Anti-Calmodulin Binding Protein-tag pAb (polyclonal)

Smart-IP series

3190	Magnetic Rack
M198-9	Anti-E-tag mAb-Magnetic beads (21D11)
M185-9	Anti-DDDDK-tag mAb-Magnetic beads (FLA-1)
D291-9	Anti-His-tag mAb-Magnetic beads (OGHis)
D153-9	Anti-GFP mAb-Magnetic beads (RQ2)
M165-9	Anti-RFP mAb-Magnetic beads (3G5)
M132-9	Anti-HA-tag mAb-Magnetic beads (5D8)
M180-9	Anti-HA-tag mAb-Magnetic beads (TANA2)
M047-9	Anti-Myc-tag mAb-Magnetic beads (PL14)
M167-9	Anti-V5-tag mAb-Magnetic beads (1H6)
D058-9	Anti-Multi Ubiquitin mAb-Magnetic beads (FK2)
M075-9	Mouse IgG1 (isotype control)-Magnetic beads
M076-9	Mouse IgG2a (isotype control)-Magnetic beads
M077-9	Mouse IgG2b (isotype control)-Magnetic beads
M081-9	Rat IgG2a (isotype control)-Magnetic beads
M198-10	Anti-E-tag mAb-Magnetic Agarose (21D11)
M185-10	Anti-DDDDK-tag mAb-Magnetic Agarose (FLA-1)
D291-10	Anti-His-tag mAb-Magnetic Agarose (OGHis)
D153-10	Anti-GFP mAb-Magnetic Agarose (RQ2)
M165-10	Anti-RFP mAb-Magnetic Agarose (3G5)
M132-10	Anti-HA-tag mAb-Magnetic Agarose (5D8)
M180-10	Anti-HA-tag mAb-Magnetic Agarose (TANA2)
M047-10	Anti-Myc-tag mAb-Magnetic Agarose (PL14)
M167-10	Anti-V5-tag mAb-Magnetic Agarose (1H6)

Protein Purification Kit

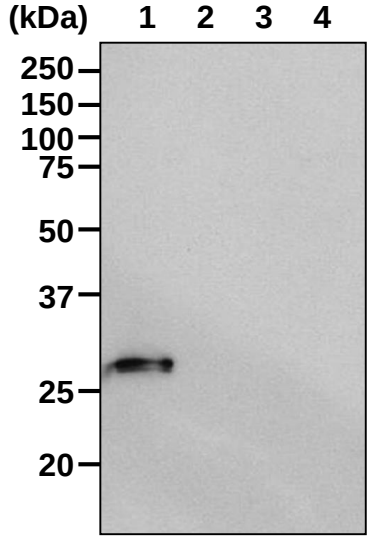
3305	c-Myc-tagged Protein MILD PURIFICATION KIT
3306	c-Myc-tagged Protein MILD PURIFICATION GEL (1 mL gel, 1 mg peptide)
3307	c-Myc-tagged Protein MILD PURIFICATION GEL (5 mL gel, 5 mg peptide)
3300-205	c-Myc tag peptide (5 mg)
3310	His-tagged Protein PURIFICATION KIT
3310-205	His-tag peptide (10 mg)
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
3320	HA-tagged Protein PURIFICATION KIT
3325	DDDDK-tagged Protein PURIFICATION 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 http://ruo.mbl.co.jp/

SDS-PAGE & Western blotting

1) Wash 1 x 10^6 cells 3 times with PBS and suspends them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 10 sec.).
2) Boil the samples for 3 min. and centrifuge. Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hr. 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 hr. at room temperature.
5) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) [5 min. x 3 times].
6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
7) Wash the membrane with PBS-T (5 min. x 3 times).
8) Incubate the membrane with the 1:10,000 of anti-IgG (Rabbit) pAb-HRP (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
9) Wash the membrane with PBS-T (5 min. x 3 times).
10) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min. 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 1 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting: 293T transfectant)



Western blot analysis of Renilla GFP

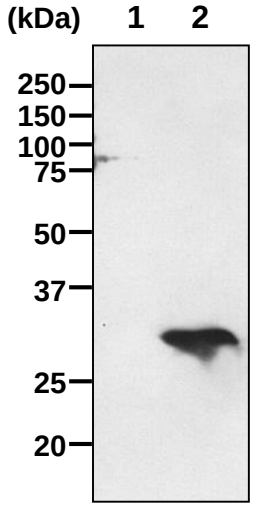
Lane 1: Renilla GFP/293T
Lane 2: 293T
Lane 3: EGFP/293T
Lane 4: *Aequorea Victria* GFP (50 ng)

Immunoblotted with Anti-Renilla GFP pAb (PM073)

Immunoprecipitation

1) Wash 1 x 10^7 cells 2 times with PBS and resuspend them with 1 mL of ice-cold Extraction buffer (50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.05% NP-40) containing appropriate protease inhibitors, then sonicate briefly (up to 15 sec.).
2) Incubate it on ice for 15min.
3) Centrifuge the tube at 12,000 x g for 10 min. at 4°C and transfer the supernatant to another tube.
4) Mix 20 μL of 50% protein A agarose beads slurry resuspended in 400 μL of IP buffer (10 mM Tris-HCl (pH 8.0), 500 mM NaCl, 0.1% NP-40) with primary antibody as suggested in the **APPLICATIONS**. Incubate with gentle agitation for 1 hr. at room temperature.
5) Wash the beads 3 times with 1 mL of IP buffer.
6) Add 500 μL of cell lysate, then incubate with gentle agitation for 1 hr. at room temperature.
7) Wash the beads 6 times with 1 mL of Extraction buffer.
8) Resuspend the beads in 20 μL of Laemmli's sample buffer, boil for 3 min. and centrifuge.
9) Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
10) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hr. 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.
11) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for overnight at 4°C.
12) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) [5 min. x 3 times].
13) Incubate the membrane with 1:1,000 of anti-Renilla GFP pAb (MBL; code no. PM073) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
14) Wash the membrane with PBS-T (5 min. x 3 times).
15) Incubate the membrane with the 1:1,000 of Rabbit TrueBlot® Anti-Rabbit IgG-HRP (eBioscience; code no. 18-8816-31) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
16) Wash the membrane with PBS-T (5 min. x 3 times).
17) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min.
18) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
19) Expose to an X-ray film in a dark room for 10 sec. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; 293T transfectant)



Immunoprecipitation of Renilla GFP from 293T transfectant

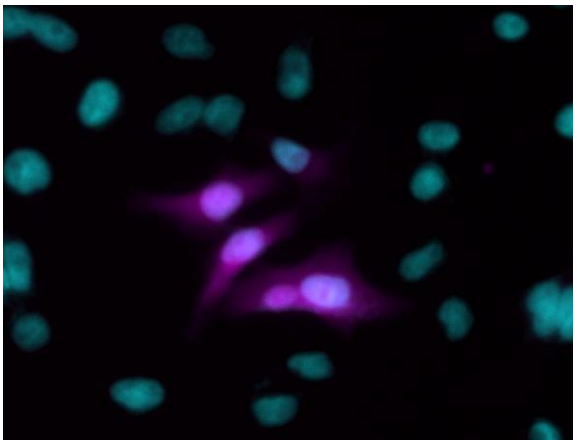
Lane 1: Normal rabbit IgG (PM035)
Lane 2: Anti-Renilla GFP pAb (PM073)

Immunoblotted with PM073

Immunocytochemistry

1) Spread the cells on a glass slide, then incubate in a CO_2 incubator for one night.
2) Remove the culture supernatant by careful aspiration.
3) Wash the slide 2 times with PBS.
4) Fix the cells with 4% paraformaldehyde (PFA)/PBS for 10 min. at room temperature (20~25°C).
5) Wash the slide 2 times with PBS.
6) Permeabilize the cells with 200 μL of 0.1% Triton X-100/PBS for 10 min. at room temperature.
7) Wash the slide 1 time with PBS.
8) Add 10% Goat serum/PBS to the cell and incubate for 5 min. at room temperature.
9) Tip off 10% Goat serum/PBS and add 200 μL of the primary antibody diluted with PBS as suggested in the **APPLICATIONS** onto the cells. Incubate for 1 hr. at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
10) Wash the slide 1 time with PBS.
11) Add 200 μL of 1:400 Alexa Fluor® 647 Goat Anti-rabbit IgG (Invitrogen; code no. A21245) diluted with PBS onto the cells. Incubate for 1 hr. at room temperature. Keep out light by aluminum foil.
12) Wash the slide 1 time with PBS.
13) Wipe excess liquid from the slide but take care not to touch the cells. Never leave the cells to dry.
14) Counterstain with DAPI for 5 min. at room temperature.
15) Wash the slide 1 time with PBS.
16) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; HeLa transfectant)



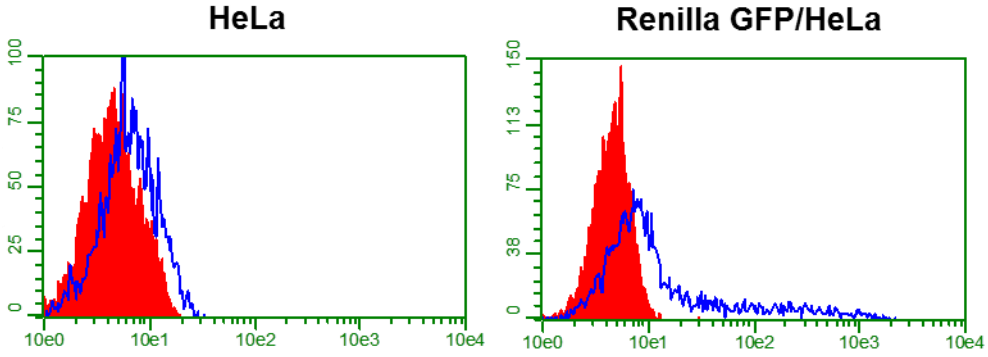
Immunocytochemical detection of Renilla GFP in HeLa transfectant

Magenta: Anti-Renilla GFP pAb (PM073)
Cyan: DAPI

Flow cytometric analysis

1) Wash 5 x 10^5cells 3 times with 1 mL of washing buffer (PBS containing 2% fetal calf serum (FCS)).
2) Add 100 µL of 4% paraformaldehyde (PFA)/PBS to the cell pellet after tapping. Mix well, then fix the cells for 10 min. at room temperature.
3) Wash the cells 2 times with 1 mL of the washing buffer.
4) Add 20 µL of 1 mg/mL Human IgG in normal goat serum to the cell pellet after tapping. Mix well and incubate for 5 min. at room temperature.
5) Add 40 µL of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted in the washing buffer. Mix well and incubate for 30 min. at room temperature.
6) Wash the cells 1 time with 1 mL of the washing buffer.
7) Add 40 µL of 1:100 anti-Rabbit IgG-PE (Beckman Coulter; code no. 732743) diluted in the washing buffer. Mix well and incubate for 30 min. at room temperature.
8) Wash the cells 1 time with 1 mL of the washing buffer.
9) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; HeLa transfectant)



Flow cytometric detection of Renilla GFP in HeLa transfectant

Open: Anti-Renilla GFP (PM073)
Closed: Normal Rabbit IgG (PM035)