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

Amalgaam
MBL
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POLYCLONAL ANTIBODY

Anti-monomeric Kusabira-Orange 2 pAb

Code No.	Quantity	Form
PM051M	100 µL	Affinity Purified

BACKGROUND: *CoralHue*™ Kusabira-Orange (KO) has been cloned from the stony coral, whose Japanese name is "Kusabira-ishi". Wild-type *CoralHue*™ KO forms a brightly fluorescent dimer. *CoralHue*™ KO has been carefully engineered to form a monomer, *CoralHue*™ monomeric Kusabira Orange 1 (mKO1) that maintains the brilliance and pH stability of the parent protein. *CoralHue*™ mKO2 is the mutant of mKO1 and has a feature of the rapid maturation. It absorbs light maximally at 551 nm and emits orange light at 565 nm. *CoralHue*™ mKO2 can be used to label proteins or subcellular structures, or for reporter assay.
PM051M is available for immunostaining of "Fucci-G_1 Orange" (Fucci; *F*luorescent *U*biquitination-based *C*ell *C*ycle *I*ndicator). Fucci-G_1 Orange encodes *CoralHue*™ monomeric Kusabira-Orange2 (mKO2) fused to a part of human Cdt1 (hCdt1: Cdc10 dependent transcript 1). It is possible to use PM051M for Fucci transgenic strain, B6.Cg-Tg(Fucci)596Bsi mice which express Fucci-G_1 Orange.

SOURCE: This antibody was purified from rabbit serum using affinity column. The rabbit was immunized with recombinant monomeric Kusabira-Orange 2.

FORMULATION: 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.

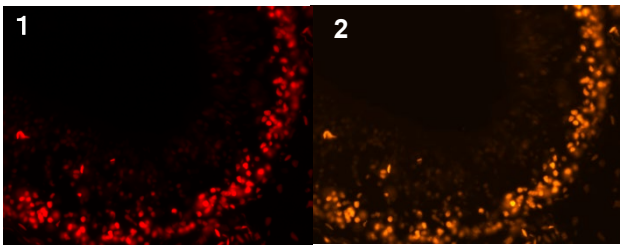
REACTIVITY: This antibody reacts with monomeric Kusabira-Orange 2 on Western blotting, Immunoprecipitation, Immunocytochemistry and Immunohistochemistry.

APPLICATIONS:
Western blotting; 1:1,000 for chemiluminescence detection system
Immunoprecipitation; 2 µL/Sample
Immunohistochemistry; 1:500 (paraffin and frozen section)
Heat treatment is necessary.
Microwave oven; 500 W in 1 mM EDTA (pH 8.0)
Frozen section: 3 minutes
Paraffin section: 10 minutes
Immunocytochemistry; 1:500
Flow cytometry; Not tested
Detailed procedure is provided in the following **PROTOCOLS**.

INTENDED USE:
For Research Use Only. Not for use in diagnostic procedures.

REFERENCES:
1) Sakaue-Sawano, A., *et al., Cell* **132**, 487-498 (2008)
2) Sakaue-Sawano, A., *et al., Chem. Biol.* **15**, 1243-1248 (2008)

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



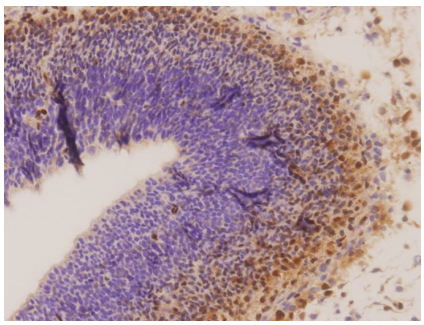
Immunohistochemical detection of mKO2 on frozen section of B6.Cg-Tg(Fucci)596Bsi mouse embryonic brain (E12) with PM051M (1) and Fucci-G_1 Orange own fluorescence (2).

Fluorescence Microscope: Axiovert200
Filter set:
1: Carl Zeiss Filter sets No.26 (for Alexa Fluor® 647)
2: FSET-KOHQ (for mKO2)
Lens: Plan-NEOFLUAR (Carl Zeiss), x20, NA=0.5

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

PROTOCOLS:
Immunohistochemical staining for frozen sections
For 4% paraform aldehyde fixed section
1) Wash the slides in PBS for 15 minutes.
2) Heat treatment
Heat treatment by Microwave:
Place the slides put on staining basket in 1 L beaker with 500 mL of 1 mM EDTA (pH 8.0). Cover the beaker with plastic wrap, then process the slides 3 minutes at 500 W with microwave oven. Let the slides cool down in the beaker at room temperature for about 40 minutes.
3) Immerse the slide in PBS containing 0.1% Tween-20 (PBS-T) for 30 minutes at room temperature.
4) Remove the slides from PBS-T, wipe gently around each section and cover tissues with blocking buffer (PBS

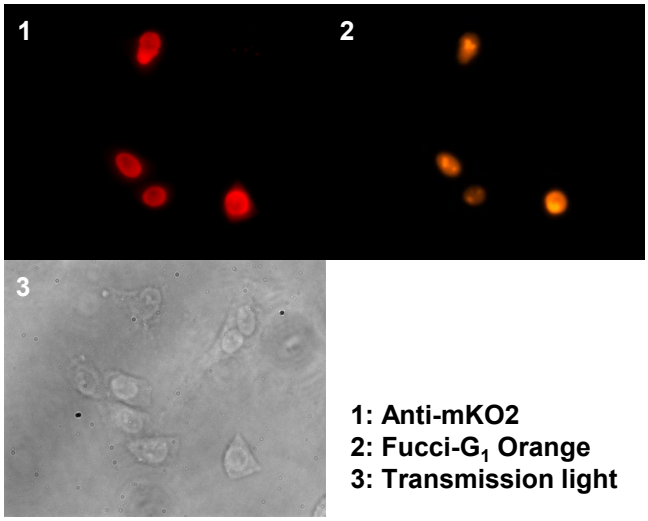
containing 2% FCS, 0.1% Tween-20) for 5 minutes to block non-specific staining. Do not wash.
5) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with blocking buffer as suggested in the **APPLICATIONS.** Incubate the sections for 1 hour at room temperature.
6) Wash the slides 2 times in PBS-T for 5 minutes each.
7) Wipe gently around each section and cover tissues with 1:500 Alexa Fluor® 647 conjugated anti-rabbit IgG (Invitrogen; code no. A21245). Incubate for 30 minutes at room temperature. Wash as in step 6).
8) Wipe excess liquid off the slide but take care not to touch the section. Never leave the section to dry.
9) Promptly add mounting medium onto the slide, then put a cover slip on it.



Immunohistochemical detection of mKO2 in paraffin embedded section of B6.Cg-Tg(Fucci)596Bsi mouse embryonic brain (E12) with PM051M.

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 3 times in PBS for 3-5 minutes each.
4) Heat treatment
   Heat treatment by Microwave:
   Place the slides put on staining basket in 500 mL beaker with 500 mL of 1 mM EDTA (pH 8.0). Cover the beaker with plastic wrap, then process the slides for 10 minutes at 500 W with microwave oven. Let the slides cool down in the beaker at room temperature for about 40 minutes.
5) Remove the slides from the 1 mM EDTA (pH 8.0) 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.
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) for 5 minutes 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**.
8) Incubate the sections for 1 hour at room temperature.
9) Wash the slides 3 times in PBS for 5 minutes each.
10) Wipe gently around each section and cover tissues with Histostar™ (Rb) for Mouse tissue (MBL; code no. 8470). Incubate for 1 hour at room temperature. Wash as in step 9).
11) Visualize by reacting for 4 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) Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each. Now ready for mounting.



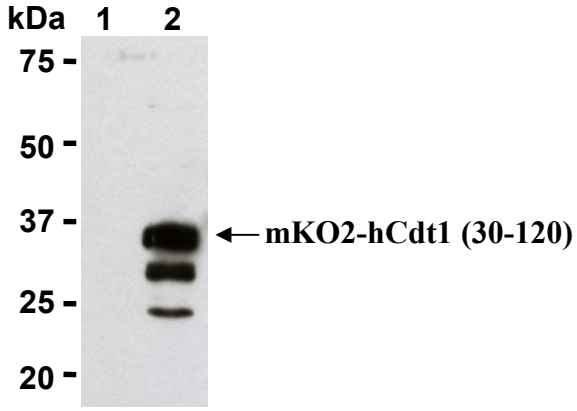
Immunocytochemical detection of mKO2 in Fucci-G_1 Orange transfected HeLa with PM051M.

Fluorescence Microscope: Axiovert200
Filter set:
1: Carl Zeiss Filter sets No.26 (for Alexa Fluor® 647)
2: FSET-KOHQ (for mKO2)
Lens: LD ACHROPLAN (Carl Zeiss), x40, NA=0.6

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (For example, spread 1 x 10^4 cells for one slide, then incubate in a CO_2 incubator for one night.)
2) Wash the glass slide 2 times with PBS.
3) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde for 10 minutes at room temperature.
4) Wash the glass slide 2 times with PBS.
5) Immerse the slide in PBS containing 0.1% Tween-20 for 30 minutes at room temperature.
6) Add the primary antibody diluted with PBS containing 0.1% Tween-20, 2% FCS as suggested in the **APPLICATIONS** onto the cells and incubate for 1 hour at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)

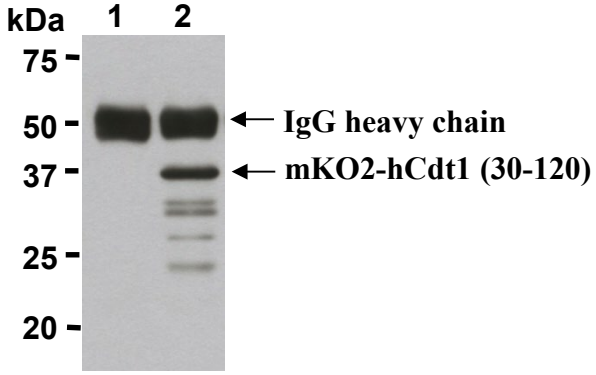
7) Wash the glass slide 2 times with PBS containing 0.1% Tween-20 (PBS-T).
8) Add 200 µL of 1:500 Alexa Fluor® 647 conjugated anti-rabbit IgG (Invitrogen; code no. A21245) diluted with PBS containing 0.1% Tween-20, 2% FCS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
9) Wash the glass slide 2 times with PBS-T.
10) Wipe excess liquid off the slide but take care not to touch the cells. Never leave the cells to dry.
11) Promptly add mounting medium onto the slide, then put a cover slip on it.



Western blot analysis in HeLa (1) and Fucci-HeLa (2) using PM051M.

SDS-PAGE & Western Blotting

1) Wash cells (approximately 1 x 10^7 cells) 3 times with PBS and resuspend them in 1 mL of Laemmli's sample buffer.
2) Boil the samples for 3 minutes and centrifuge. Load 10 µL of sample 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 for 1 hour at room temperature with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS**. (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 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 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 a paper towel, and seal it in plastic wrap.
11) Expose the membrane onto an X-ray film in a dark room for 3 minutes. Develop the film under usual settings. The conditions for exposure and development may vary.



Immunoprecipitation of mKO2 from Fucci-G$_1$ Orange transfected 293T with normal rabbit IgG (1) or PM051M (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with PM051M.*

Immunoprecipitation

1) Wash cells (approximately 1 x 10^7 cells) 3 times with PBS and resuspend them in 1 mL of cold Lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.05% NP-40) containing protease inhibitors at appropriate concentrations. Incubate it at 4°C with rotating for 30 minutes; thereafter, briefly sonicate the mixture (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 primary antibody as suggested in the **APPLICATIONS** into 200 µL of the supernatant. 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 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 agarose with cold Lysis buffer.
7) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
8) Repeat steps 6)-7) 2-4 times.
9) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 20 µL/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)

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CoralHue™ **mKO** is a product of co-development with Dr. Atsushi Miyawaki at the Laboratory for Cell Function and Dynamics, the Brain Science Institute, and the Institute of Physical and Chemical Research (RIKEN).