

POLYCLONAL ANTIBODY

Anti-monomeric Azami-Green 1 pAb

Code No.	Quantity	Form
PM052M	100 μ L	Affinity Purified

BACKGROUND: *CoralHue*[®] Azami-Green (AG) has been cloned from the stony coral, whose Japanese name is "Azami-Sango". It absorbs light maximally at 492 nm and emits green light at 505 nm. Wild-type *CoralHue*[®] AG rapidly matures to form a tetrameric complex. *CoralHue*[®] AG has been carefully engineered to form a monomer, *CoralHue*[®] monomeric Azami-Green (mAG1) that maintains the brilliance and pH stability of the parent protein. *CoralHue*[®] mAG1 can be used to label proteins or subcellular structures. *CoralHue*[®] hmAG1 sequence is codon-optimized for higher expression in mammalian cells. PM052 is available for immunostaining of "Fucci-S/G₂/M Green" (Fucci; Fluorescent Ubiquitination-based Cell Cycle Indicator). Fucci-S/G₂/M Green encodes *CoralHue*[®] humanized monomeric Azami-Green1 (hmAG1) fused to a part of human Geminin (hGeminin). It is possible to use PM052 for Fucci transgenic strain, B6.Cg-Tg(Fucci)504Bsi mice which express Fucci-S/G₂/M Green.

SOURCE: This antibody was purified from rabbit serum using affinity column. The rabbit was immunized with recombinant *CoralHue*[®] monomeric Azami-Green 1.

FORMULATION: 100 μ L 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 *CoralHue*[®] monomeric Azami-Green 1 on Western blotting, Immunoprecipitation, Immunocytochemistry and Immunohistochemistry.

APPLICATIONS:

Western blotting; 1:1,000

Immunoprecipitation; 2 μ L/Sample

Immunohistochemistry; 1:100 (paraffin and frozen section)

Heat treatment is necessary.

Microwave oven; 500 W in 1 mM EDTA (pH 8.0)

Treatment time for frozen sections: 3 minutes

for paraffin sections: 5 minutes*

*Please be sure to microwave just for 5 minutes.

10 minutes treatment causes no staining.

Immunocytochemistry; 1:100

Flow cytometry; Not tested

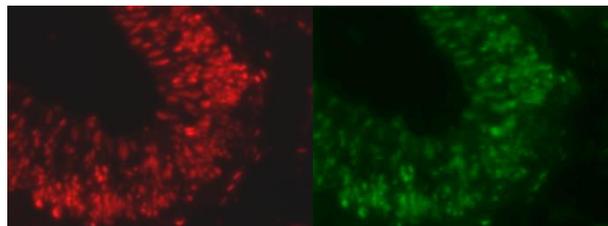
Detailed procedure is provided in the following **PROTOCOLS**.

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)



Immunohistochemical detection of mAG1 on frozen section of B6.Cg-Tg(Fucci)504Bsi mouse embryonic brain (E13) with PM052M (1) and Fucci-S/G₂/M Green own fluorescence (2).

Fluorescence Microscope: Axiovert200

Filter set:

- 1: Carl Zeiss Filter sets No.26 (for Alexa Fluor[®] 647)
- 2: U-MGFPHQ (for mAG1)

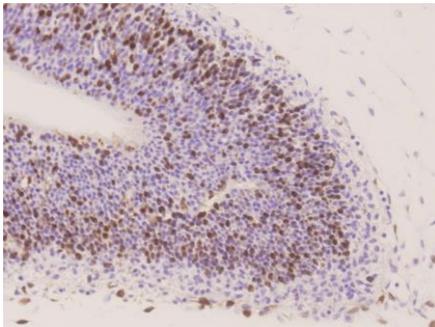
Lens: Plan-NEOFLUAR (Carl Zeiss), x20, NA=0.5

PROTOCOLS:

Immunohistochemical staining for frozen sections and 4% paraformaldehyde fixed section

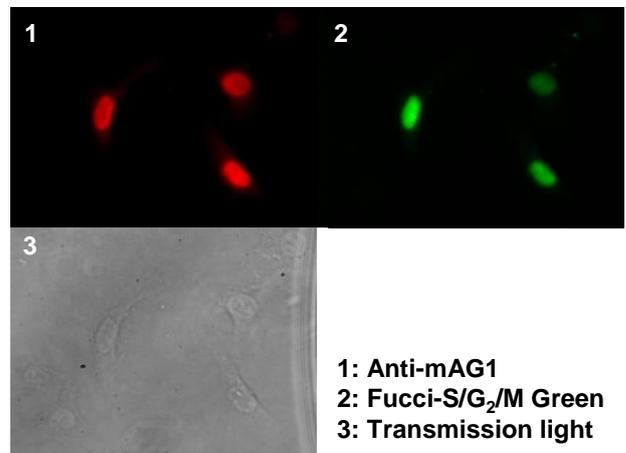
- 1) Wash the slides in PBS for 15 minutes.
- 2) Heat treatment
Heat treatment by Microwave:
Place the slides put on staining basket in 1 L beaker with 500 mL of 1 mM EDTA (pH 8.0). Cover the beaker with plastic wrap, then process the slides 3 minutes at 500 W with microwave oven. Let the slides cool 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**.
- 6) Incubate the sections for 1 hour at room temperature.
- 7) Wash the slides twice in PBS-T for 5 minutes each.
- 8) Wipe gently around each section and cover tissues with 1:500 Alexa Fluor® 647 conjugated anti-rabbit IgG (Thermo Fisher Scientific, code no. A21245). Incubate for 30 minutes at room temperature. Wash as in step 7).
- 9) Wipe excess liquid off the slide but take care not to touch the section. Never leave the section to dry.
- 10) Promptly add mounting medium onto the slide, then put a cover slip on it.



Immunohistochemical detection of mAG1 in paraffin embedded section of B6.Cg-Tg(Fucci)504Bsi mouse embryonic brain (E13) with PM052M.

- 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 ENVISION+Dual Link (Agilent, code no. K4063). Incubate for 1 hour at room temperature. Wash as in step 9).
- 11) Visualize by reacting for 5 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.



1: Anti-mAG1
2: Fucci-S/G₂/M Green
3: Transmission light

Immunocytochemical detection of mAG1 in Fucci-S/G₂/M Green transfected HeLa with PM052M.

Fluorescence Microscope: Axiovert200

Filter set:

1: Carl Zeiss Filter sets No.26 (for Alexa Fluor® 647)

2: U-MGFPHQ (for mAG1)

Lens: Plan-ACHROPLAN (Carl Zeiss), x40, NA=0.6

Immunocytochemistry

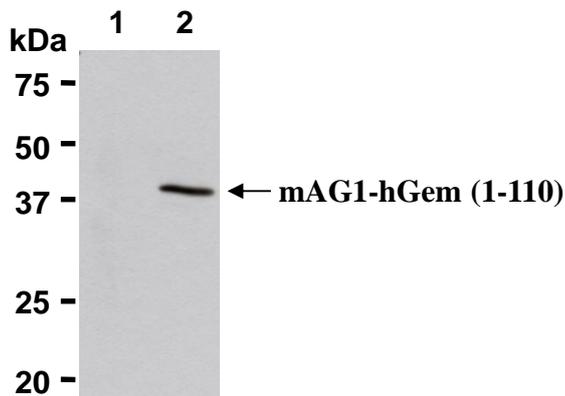
- 1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 1×10^4 cells for one slide, then incubate in a CO₂ incubator overnight.)
- 2) Wash the glass slide twice with PBS.
- 3) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde for 10 minutes at room temperature.
- 4) Wash the glass slide twice 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 are recommended if necessary.)
- 7) Wash the glass slide twice with PBS containing 0.1% Tween-20 (PBS-T).

Immunohistochemical staining for paraffin-embedded sections

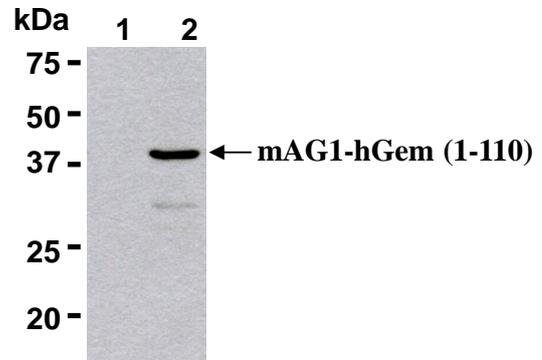
- 1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
- 2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
- 3) Wash the slides 3 times in PBS for 3-5 minutes each.
- 4) Heat treatment
Heat treatment by Microwave:
Place the slides put on staining basket in 1 L beaker with 500 mL of 1 mM EDTA (pH 8.0). Cover the beaker with plastic wrap, then process the slides for 5 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% H₂O₂ 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) Add 200 μ L of 1:500 Alexa Fluor[®] 647 conjugated anti-rabbit IgG (Thermo Fisher Scientific, code no. A21245) diluted with PBS containing 0.1% Tween-20, 2% FCS onto the cells.
- 9) Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
- 10) Wash the glass slide twice with PBS-T.
- 11) Wipe excess liquid off the slide but take care not to touch the cells. Never leave the cells to dry.
- 12) Promptly add mounting medium onto the slide, then put a cover slip on it.

- 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.



Western blot analysis in HeLa (1) and Fucci-HeLa (2) using PM052M.



Immunoprecipitation of mAG1 from Fucci-S/G₂/M Green transfected 293T with normal rabbit IgG (1) or PM052M (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with Anti-monomeric Azami-Green 1 mAb (MBL, code no. M102-3M).

SDS-PAGE & Western blotting

- 1) Wash cells (approximately 1×10^7 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 μ L 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/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 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).
- 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).
- 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.

Immunoprecipitation

- 1) Wash cells (approximately 1×10^7 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 μ L of the supernatant. Mix well and incubate with gentle agitation for 60-120 minutes at 4°C. Add 20 μ L 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.
- 4) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
- 5) Resuspend the agarose with cold Lysis buffer.
- 6) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
- 7) Repeat steps 5)-6) 2-4 times.
- 8) Resuspend the beads in 20 μ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
- 9) Load 20 μ L of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
- 10) 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.
- 11) 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.

- 12) Incubate the membrane for 1 hour at room temperature with 1:1,000 Anti-monomeric Azami-Green 1 mAb (MBL, code no. M102-3M) as primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2). (The concentration of antibody will depend on the conditions.)
- 13) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3). Incubate the membrane with 1:10,000 Anti-IgG (Mouse) pAb-HRP (MBL, code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 14) Wash the membrane with PBS-T (5 minutes x 3).
- 15) Wipe excess buffer off the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute.
- 16) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
- 17) 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.

RELATED PRODUCTS:

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CoralHue[®] mAG 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).

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