

POLYCLONAL ANTIBODY

Anti-Azami-Green pAb

Code No.	Quantity	Form
PM011M	100 µL	Purified IgG

BACKGROUND: The fluorescent protein, *CoralHue™* Azami-Green (AG) from the stony coral, whose Japanese name is “Azami-Sango”. It absorbs light maximally at 492 nm and emits green light at 505 nm. While AG forms a tetrameric complex, it matures rapidly to be fluorescent. AG has been carefully engineered to form a monomer, *CoralHue™* monomeric Azami-Green 1 (mAG1) that maintains the brightness and pH stability of the parent protein.

SOURCE: This antibody was fractionated by salting out and gel filtration on a Sephadex G-200 column. The rabbit was immunized with the recombinant *CoralHue™* monomeric Azami-Green 1.

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 both of tetramer and monomer form of *CoralHue™* Azami-Green on Western blotting.

APPLICATIONS:

Western blotting; 1:1,000

Immunoprecipitation; Not tested

Immunohistochemistry; Not tested

Immunocytochemistry; Not tested

Flow cytometry; Not tested

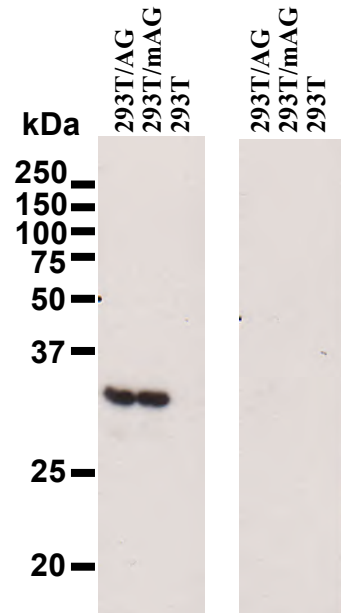
Detailed procedure is provided in the following **PROTOCOL**.

INTENDED USE:

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

REFERENCE:

1) Karasawa, S., *et al.*, *J. Biol. Chem.* **278**, 34167-34171 (2003)



Western blotting detection of Azami-Green (AG) or monomer Azami-Green (mAG) expressed in 293T cells.

Left; Immunoblotted with PM011M

Right; Immunoblotted with Normal rabbit IgG

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

PROTOCOL:

SDS-PAGE & Western blotting

- 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. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.
- 3) Mix the sample with equal volume of Laemmli's sample buffer.
- 4) 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.
- 5) 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.

- 6) 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.
- 7) 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.)
- 8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
- 9) Incubate the membrane with the 1:10,000 anti-IgG (H+L chain) pAb (MBL, code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 10) Wash the membrane with PBS-T (10 minutes x 3).
- 11) Wipe excess buffer off the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
- 12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 13) Expose to an X-ray film in a dark room for 3 minutes.
- 14) Develop the film as usual. The condition for exposure and development may vary.

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