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

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MONOCLONALANTIBODY

Anti-monomeric Azami-Green 1 mAb

Code No.	Clone	Subclass	Quantity	Concentration
M102-3M	2F11	Mouse IgG1 κ	100 µL	1 mg/mL

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 purified from hybridoma (clone 2F11) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with C3H mouse lymphocyte immunized with the recombinant *CoralHue*® monomeric Azami-Green 1.

FORMULATION: 100 µg IgG in 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 *CoralHue*® monomeric Azami-Green 1 on Western blotting.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not recommended*
*Clone 3D10 is suitable for this application. Please refer to the data sheet (MBL, code no. M103-3M).
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

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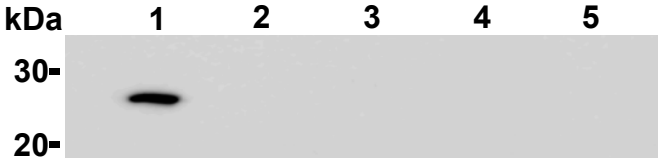
REFERENCE:
1) Karasawa, S., *et al.*, *J. Biol. Chem.* **278**, 34167-34171 (2003)



Western blot analysis of Azami-Green (1), Kusabira-Orange (2), Kaede (3), Dronpa (4) and Midoriishi-Cyan (5) recombinant protein using M102-3M.

The descriptions of the following protocols are examples.

Each user should determine the appropriate condition.

PROTOCOL:

SDS-PAGE & Western Blotting

1) Mix the recombinant *CoralHue*® monomeric Azami-Green 1 with equal volume of Laemmli's sample buffer.
2) Boil the samples for 2 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 10 V for 45 minutes 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 100% Block Ace 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 10% Block Ace 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 6 times).
7) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG (BioRad; code no. 170-6516) diluted with 1% BSA (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 on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
10) Expose to an X-ray film in a dark room for 2 minutes. Develop the film as usual. The condition 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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Patent Nos. JP4214209, US7247449 and EP1452591.