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

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MONOCLONAL ANTIBODY

Anti-Midoriishi-Cyan mAb

Code No.	Clone	Subclass	Quantity	Concentration
M130-3M	5B7	Mouse IgG1	100 µL	1 mg/mL

BACKGROUND: The fluorescent protein, *CoralHue*™ Midoriishi-Cyan 1 (MiCy1), from the stony coral whose Japanese name is “Midori-ishi”. It absorbs light maximally at 472 nm and emits cyan light at 495 nm. Wild-type MiCy1 rapidly matures to form a fluorescent dimeric complex. MiCy1 can be used to mark individual cells or to report gene expression without problems stemming from protein aggregation.

SOURCE: This antibody was purified from hybridoma (clone 5B7) supernatant using protein A column. This hybridoma was established by fusion of mouse myeloma cell P3U1 with C3H mouse lymphocyte immunized with the recombinant *CoralHue*™ Midoriishi-Cyan 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*™ Midoriishi-Cyan 1 and *CoralHue*™ monomeric Midoriishi-Cyan 1 on Western blotting.

APPLICATIONS:

Western blotting; 1 µg/mL
Immunoprecipitation; Not recommended
*Clone 2C1 is suitable for this application. Please refer to the data sheet (MBL, code no. M116-3M).
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.*, *Biochem J.* **381**, 307-312 (2004)

Western blotting analysis of Azami-Green (1), Dronpa-Green (2), Kaede (3), Keima-Red (4), Kikume Green-Red (5), Kusabira-Orange (6) and Midoriishi-Cyan (7) from E. coli using M130-3M.

PROTOCOL:

SDS-PAGE & Western blotting

1) Mix the sample with equal volume 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 polvinylidene 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 with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk 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 (5 minutes x 6 times).
7) 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.
8) Wash the membrane with PBS (5 minutes x 6 times).
9) Wipe excess buffer off 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 20 seconds. Develop the film as usual. The condition for exposure and development may vary.

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