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

Amalgaam MBL

MONOCLONAL ANTIBODY

Anti-Midoriishi-Cyan mAb

Code No.	Clone	Subclass	Quantity	Concentration
M116-3M	2C1	Mouse IgG2b	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 2C1) 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 on Immunoprecipitation.

APPLICATIONS:

Western blotting; Not recommended
*Clone 5B7 is suitable for this application. Please refer to the data sheet (MBL, code no. M130-3M).
Immunoprecipitation; 2 µg/sample
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

INTENDED USE:

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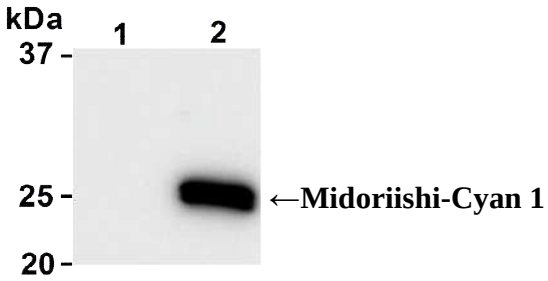
REFERENCE:

1) Karasawa, S., *et al.*, *Biochem J.* **381**, 307-312 (2004)



Immunoprecipitation of recombinant His tagged Midoriishi-Cyan 1 protein using mouse IgG2b isotype control (1) or M116-3M (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with anti-His-tag polyclonal antibody.*

PROTOCOL:

Immunoprecipitation

1) Add primary antibody as suggested in the **APPLICATIONS** into 30 µL of 50% protein A agarose beads resuspended in the cold Lysis buffer (50 mM Tris-HCl pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol). Mix well and incubate with gentle agitation for 60 minutes at 4°C.
2) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
3) Add 100 µL of the recombinant protein. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) Resuspend the beads in 20 µL of Laemmli's sample buffer and 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.
6) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 200 mA for 1 hour 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.
7) 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.
8) Incubate the membrane with the 1:1,000 Anti-His-tag pAb (MBL; code no. PM032) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
9) Wash the membrane with PBS (5 minutes x 6 times).
10) Incubate the membrane with 1:10,000 Anti-IgG (Rabbit) pAb-HRP (MBL; code no. 458) diluted with 1% BSA for 1 hour at room temperature.

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URL http://ruo.mbl.co.jp/
e-mail support@mbl.co.jp, TEL 052-238-1904

11) 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.
12) 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:

PM011M	Anti-Azami-Green pAb (polyclonal)
M103-3M	Anti-Azami-Green mAb (3D10)
PM052M	Anti-monomeric Azami-Green 1 pAb (polyclonal)
M104-3M	Anti-monomeric Kusabira-Orange 1 mAb (1H7)
M105-3M	Anti-monomeric Kusabira-Orange 1 mAb (2G9)
M168-3M	Anti-monomeric Kusabira-Orange 2 mAb (3B3)
PM051M	Anti-monomeric Kusabira-Orange 2 pAb (polyclonal)
M126-3M	Anti-monomeric Keima-Red mAb (2F7)
M148-3M	Anti-monomeric Kusabira-Green N-terminal fragment mAb (1E6)
M149-3M	Anti-monomeric Kusabira-Green C-terminal fragment mAb (21B10)
M127-3M	Anti-Keima-Red mAb (3C9)
M116-3M	Anti-Midoriishi-Cyan mAb (2C1)
M130-3M	Anti-Midoriishi-Cyan mAb (5B7)
PM012M	Anti-Kaede pAb (polyclonal)
M106-3M	Anti-Kaede mAb (2F4)
M125-3M	Anti-Kaede mAb (3B1)
M128-3M	Anti-Kikume Green-Red mAb (5B3)
M129-3M	Anti-Kikume Green-Red mAb (2D3)
M117-3M	Anti-Dronpa-Green mAb (4D12)
M118-3M	Anti-Dronpa-Green mAb (2F6)
598	Anti-GFP (Green Fluorescent Protein) pAb (polyclonal)
598-7	Anti-GFP pAb-HRP-DirecT (polyclonal)
M048-3	Anti-GFP mAb (1E4)
D153-3	Anti-GFP mAb (RQ2)
D153-6	Anti-GFP mAb-Biotin (RQ2)
D153-8	Anti-GFP mAb-Agarose (RQ2)
D153-11	Anti-GFP mAb-Magnetic Beads (RQ2)
D153-10	Anti-GFP mAb-Magnetic Agarose (RQ2)
D153-A48	Anti-GFP mAb-Alexa Fluor® 488 (RQ2)
D153-A59	Anti-GFP mAb-Alexa Fluor® 594 (RQ2)
D153-A64	Anti-GFP mAb-Alexa Fluor® 647 (RQ2)
PM073	Anti-Renilla GFP pAb (polyclonal)
PM005	Anti-RFP pAb (polyclonal)
PM005-7	Anti-RFP pAb-HRP-DirecT (polyclonal)
M155-3	Anti-RFP mAb (8D6)
M165-3	Anti-RFP mAb (3G5)
M165-8	Anti-RFP mAb-Agarose (3G5)
M165-11	Anti-RFP mAb-Magnetic Beads (3G5)
M165-10	Anti-RFP mAb-Magnetic Agarose (3G5)
M204-3	Anti-RFP mAb (1G9)
M204-7	Anti-RFP mAb-HRP-DirecT (1G9)
M208-3	Anti-RFP mAb Cocktail (1G9, 3G5)

***CoralHue*® MiCy1** 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 No. JP4852676.