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

Amalgaam
MBL

MONOCLONALANTIBODY

Anti-monomeric Kusabira-Orange 1 mAb

Code No.	Clone	Subclass	Quantity	Concentration
M104-3M	1H7	Mouse IgG1 κ	100 µL	1 mg/mL

BACKGROUND: The *CoralHue*® "Kusabira-Orange (KO)" which was cloned from the stony coral whose Japanese name is "Kusabira-ishi". KO absorbs light maximally at 548 nm and emits orange light at 561 nm. Wild-type KO rapidly matures to form a fluorescent dimeric complex. KO has been carefully engineered to form a monomer, *CoralHue*® monomeric Kusabira Orange 1 (mKO1) that maintains the brilliance and pH stability of the parent protein. mKO1 can be used to label proteins or subcellular structures or for FRET analysis.

SOURCE: This antibody was purified from hybridoma (clone 1H7) 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 Kusabira-Orange 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 Kusabira-Orange 1, *CoralHue*® monomeric Kusabira-Orange 2 and *CoralHue*® monomeric Kusabira-Green on Western blotting.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not recommended
*Clone 2G9 is suitable for this application. Please refer to the data sheet (MBL, code no. M105-3M).
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

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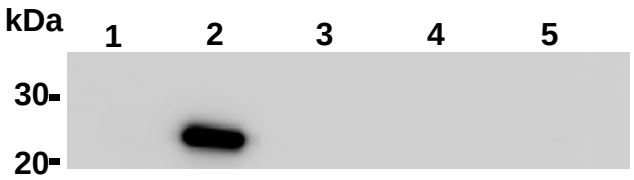
REFERENCE:
1) Karasawa, S., *et al.*, *Biochem J.* **381**, 307-312 (2004)



Western blot analysis of Azami-Green (1), mKO1 (2), Kaede (3), Dronpa (4) and Midoriishi-Cyan (5) recombinant protein using M104-3M.

PROTOCOL:

SDS-PAGE & Western Blotting

1) Mix the recombinant protein 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, pH7.2 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 (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 (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 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)

CoralHue® **mKO** 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).

Patent Nos. JP4258724, US7226993 and EP1700913 and patents pending.