

MONOCLONALANTIBODY

Anti-monomeric Kusabira-Orange 1 mAb

Code No.	Clone	Subclass	Quantity	Concentration
M104-3M	1H7	Mouse IgG1 κ	100 μ L	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 μ g IgG in 100 μ L 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 μ g/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:

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

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 μ L 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).

Use of **CoralHue[®] mKO** requires a license from MBL Co., Ltd. MBL grants non-profit research organizations the right to use the product for non-commercial research purposes. For commercial entities a commercial license is required.

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