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

Amalgaam MBL

MONOCLONALANTIBODY

Anti-Kaede mAb

Code No.	Clone	Subclass	Quantity	Concentration
M106-3M	2F4	Mouse IgG1 κ	100 µL	1 mg/mL

BACKGROUND: *CoralHue*® Kaede protein emits green fluorescence that can be converted to red. The red fluorescence is comparable in intensity to the green and is stable under usual aerobic conditions. The green-to-red conversion is highly sensitive to irradiation with UV or violet light (350-410 nm). Maximal illumination results in a 2,000-fold increase in the ratio of red-to-green signal. The excitation lights used to elicit red and green fluorescence do not induce the photoconversion. This property provides a simple and powerful technique for regional optical marking.

SOURCE: This antibody was purified from hybridoma (clone 2F4) 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*® Kaede.

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 clearly immunoprecipitates *CoralHue*® Kaede. But it slightly immunoprecipitates *CoralHue*® Midoriishi-Cyan 1 and *CoralHue*® Dronpa-Green 1.

APPLICATIONS:

Western blotting; Not recommended*
*Clone 3B1 is suitable for this application. Please refer to the data sheet (MBL code no. M125-3M).
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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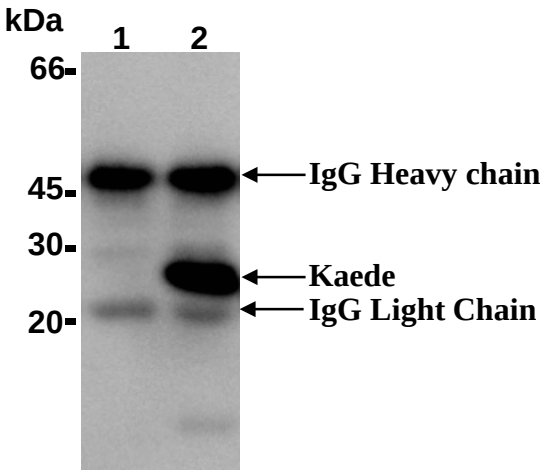
REFERENCE:

1) Ando, R., *et al.*, *PNAS* **99**, 12651-12656 (2002)



Immunoprecipitation of recombinant His tagged Kaede using mouse IgG1 isotype control (1) or M106-3M (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 suggest 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 1 hour 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 1 hour 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 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.
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.

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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-T [0.05% Tween-20 in PBS] (5 minutes x 6 times).
10) Incubate the membrane with the 1:10,000 HRP-conjugated anti-rabbit IgG (BioRad; code no. 170-6515) diluted with 1% BSA (in PBS, pH 7.2) for 1 hour at room temperature.
11) Wash the membrane with PBS-T (5 minutes x 6 times).
12) 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.
13) Expose to an X-ray film in a dark room for 5 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*® Kaede** 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. JP5117464 and US7345157.