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

Amalgaam MBL
A JSR Life Sciences Company

MONOCLONALANTIBODY

Anti-Kaede mAb

Code No.	Clone	Subclass	Quantity	Concentration
M125-3M	3B1	Mouse IgG1	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 3B1) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c 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 reacts with *CoralHue*™ Kaede on Western blotting.

APPLICATIONS:

Western blotting; 1 µg/mL
Immunoprecipitation; Not recommended
*Clone 2F4 is suitable for this application. Please refer to the data sheet (MBL code no. M106-3M).
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

INTENDED USE:

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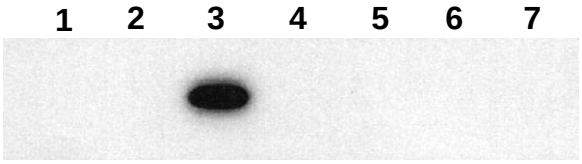
REFERENCE:

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



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 M125-3M.

PROTOCOL:

SDS-PAGE & Western blotting

1) Mix the sample 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% methanol). See the manufacturer's manual for precise transfer procedure.
4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk 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-T [0.05% Tween-20 in 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-T (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.

200414-2

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).
Use of ***CoralHue***™ **Kaede** 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. For more information, please contact support@mbl.co.jp.
Patent Nos. JP5117464 and US7345157.