

MONOCLONAL ANTIBODY

Anti-Kikume Green-Red mAb

Code No.	Clone	Subclass	Quantity	Concentration
M128-3M	5B3	Mouse IgG2b	100 μ L	1 mg/mL

BACKGROUND: *CoralHue*[®] Kikume Green-Red 1 (KikGR1) protein emits bright green fluorescence that can be irreversibly converted to red. The red fluorescence is comparable in intensity to the green fluorescence and is stable under usual aerobic conditions. This green-to-red photoconversion is highly sensitive to irradiation with UV or violet light (350-410 nm). Maximal illumination results in a robust increase in the ratio of red-to-green signal. The excitation lights used to elicit red or 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 5B3) supernatant using protein A column. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse lymphocyte immunized with the recombinant *CoralHue*[®] Kikume Green-Red 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*[®] Kikume Green-Red 1 and *CoralHue*[®] monomeric Kikume Green-Red 1 on Western blotting.

APPLICATIONS:

Western blotting: 1 μ g/mL

Immunoprecipitation: Not recommended

*Clone 2D3 is suitable for this application. Please refer to the data sheet (MBL, code no. M129-3M).

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) Tsutsui, H., *et al.*, *EMBO Rep.* **6**, 233-238 (2005)

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Western blotting analysis of Azami-Green (1), Dronpa-Green (2), Kaede (3), Keima-Red (4), Kikme Green-Red (5), Kusabira-Orange (6) and Midoriishi-Cyan (7) from *E. coli* using M128-3.

PROTOCOL:

SDS-PAGE & Western blotting

- 1) Mix the sample with equal volume of Laemmli's sample buffer.
- 2) Boil the samples for 3 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 1 mA/cm² for 1 hour 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 (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
- 5) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) 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).
- 7) Incubate the membrane with the 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 (5 minutes x 6).
- 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.

RELATED PRODUCTS:

- PM011M Anti-Azami-Green pAb (polyclonal)
- PM052M Anti-monomeric Azami-Green 1 pAb (polyclonal)
- M104-3M Anti-monomeric Kusabira-Orange 1 mAb (1H7)
- 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)
- 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)
- M118-3M Anti-Dronpa-Green mAb (2F6)

For more information, please visit our web site <https://ruo.mbl.co.jp/>.