

MONOCLONAL ANTIBODY

Anti-monomeric Kusabira-Green C-terminal fragment mAb

| Code No. | Clone | Subclass | Quantity | Concentration |
|----------|-------|-------------|----------|---------------|
| M149-3M | 21B10 | Mouse IgG2a | 100 µL | 1 mg/mL |

BACKGROUND: *CoralHue*[®] Fluo-chase Kit can detect protein-protein interactions as fluorescent signals using the protein fragment complementation method. The gene of *CoralHue*[®] monomeric Kusabira-Green (mKG), a reporter protein, is divided into two fragments (*CoralHue*[®] mKG_N fragment and *CoralHue*[®] mKG_C fragment) which are respectively fused to the target protein genes to investigate the interactions. When the expressed target proteins don't interact, *CoralHue*[®] mKG_N fragment and *CoralHue*[®] mKG_C fragment cannot approach each other and can not emit fluorescence. However, when target proteins interact, divided *CoralHue*[®] mKG fragments spatially approach each other and the local effective concentration increases. As a result, *CoralHue*[®] mKG fragments form a steric structure before dividing and the chromophore emits fluorescence. The fluorescent signals can be detected depending on the fused target protein-protein interactions. Clone 21B10 has the epitope on *CoralHue*[®] mKG_C fragment and can detect the fusion protein with *CoralHue*[®] mKG_C specifically.

SOURCE: This antibody was purified from hybridoma (clone 21B10) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with C3H mouse lymphocyte immunized with the *CoralHue*[®] mKG_C protein (51 aa).

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*[®] mKG_C fragment and *CoralHue*[®] mKO2 on Western blotting.

INTENDED USE:

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

REFERENCES:

- 1) Nitta, S., *et al.*, *Hepatology* **57**, 46-58 (2013) [IC]
- 2) Ono, T., *et al.*, *J Biol. Chem.* **287**, 6810-6818 (2012) [IC]

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system

Immunoprecipitation; Not tested

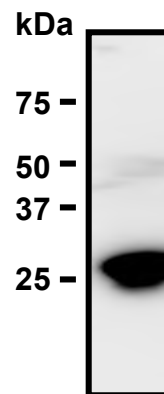
Immunohistochemistry; Not tested

Immunocytochemistry; Not tested*

*It is reported that this antibody is used for Immunocytochemistry in the reference number 1)-2).

Flow cytometry; Not tested

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



Western blot analysis of monomeric Kusabira-Green C-terminal fragment fusion protein expressed in 293T cells using M149-3M.

PROTOCOL:

SDS-PAGE & Western Blotting

- 1) Wash the 2×10^5 cells 3 times with PBS and suspend with 1 mL 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 1 mA/cm² for 1 hour 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 4% Block Ace for 1 hour at room temperature, or overnight at 4°C.
- 5) Incubate the membrane with primary antibody diluted with 0.4% 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-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 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-T (5 minutes x 3 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.

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|---------|-------------------------------------|
| M155-3 | Anti-RFP mAb (8D6) |
| M165-3 | Anti-RFP mAb (3G5) |
| M165-8 | Anti-RFP mAb-Agarose (3G5) |
| M165-9 | Anti-RFP mAb-Magnetic beads (3G5) |
| M165-10 | Anti-RFP mAb-Magnetic Agarose (3G5) |
| M204-3 | Anti-RFP mAb (1G9) |
| M204-7 | Anti-RFP mAb-HRP-Direct (1G9) |
| M208-3 | Anti-RFP mAb Cocktail (1G9, 3G5) |

RELATED PRODUCTS:

| | |
|----------|---|
| AM-1100M | <i>CoralHue</i> [®] Fluo-chase Kit |
| M148-3M | Anti-monomeric Kusabira-Green N-terminal fragment mAb (1E6) |
| M149-3M | Anti-monomeric Kusabira-Green C-terminal fragment mAb (21B10) |
| PM011M | Anti-Azami-Green pAb (polyclonal) |
| M103-3M | Anti-Azami-Green mAb (3D10) |
| PM052M | Anti-monomeric Azami-Green 1 pAb (polyclonal) |
| M102-3M | Anti-monomeric Azami-Green 1 mAb (2F11) |
| 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) |
| 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) |
| 598 | Anti-GFP (Green Fluorescent Protein) pAb (polyclonal) |
| 598-7 | Anti-GFP pAb-HRP-Direct (polyclonal) |
| M048-3 | Anti-GFP mAb (1E4) |
| D153-3 | Anti-GFP mAb (RQ2) |
| D153-6 | Anti-GFP mAb-Biotin (RQ2) |
| D153-8 | Anti-GFP mAb-Agarose (RQ2) |
| D153-9 | Anti-GFP mAb-Magnetic beads (RQ2) |
| D153-10 | Anti-GFP mAb-Magnetic Agarose (RQ2) |
| D153-A48 | Anti-GFP mAb-Alexa Fluor [®] 488 (RQ2) |
| D153-A59 | Anti-GFP mAb-Alexa Fluor [®] 594 (RQ2) |
| D153-A64 | Anti-GFP mAb-Alexa Fluor [®] 647 (RQ2) |
| PM073 | Anti-Renilla GFP pAb (polyclonal) |
| PM005 | Anti-RFP pAb (polyclonal) |
| PM005-7 | Anti-RFP pAb-HRP-Direct (polyclonal) |

CoralHue[®] **mKG** 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*[®] **mKG** 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.