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

Amalgaam MBL

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.

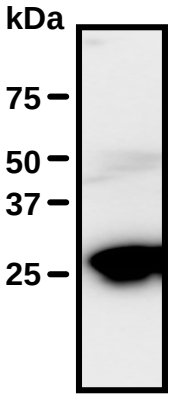
REACTIVITY: This antibody reacts with *CoralHue*® mKG_C fragment and *CoralHue*® mKO2 on Western blotting.

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 $2x10^5$ 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/cm^2 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.

RELATED PRODUCTS:

AM-1100M	*CoralHue*® 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)
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)

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).

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