

MONOCLONAL ANTIBODY

Anti-monomeric Kusabira-Green N-terminal fragment mAb

Code No.	Clone	Subclass	Quantity	Concentration
M148-3M	1E6	Mouse IgG2b	100 μ L	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, 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 1E6 has the epitope on *CoralHue*[®] mKG_N fragment and can detect the fusion protein with *CoralHue*[®] mKG_N specifically.

SOURCE: This antibody was purified from hybridoma (clone 1E6) 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_N protein (168 aa).

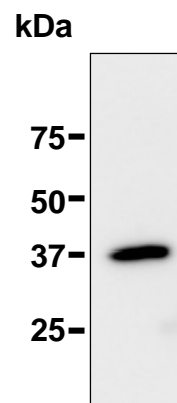
FORMULATION: 100 μ g IgG in 100 μ L volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

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*[®] mKG_N fragment and *CoralHue*[®] mKO1 and *CoralHue*[®] mKO2 on Western blotting.

INTENDED USE:
For Research Use Only. Not for use in diagnostic procedures.

REFERENCE:
1) Nitta, S., *et al. Hepatology* **57**, 46-58 (2013) [IC]



Western blot analysis of monomeric Kusabira-Green N-terminal fragment fusion protein expressed in 293T cells using M148-3M.

APPLICATIONS:

Western blotting; 1 μ g/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).

Flow cytometry; Not tested

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

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 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% 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:

- 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)

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).

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