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

Amalgaam MBL
A JSR Life Sciences Company

MONOCLONAL ANTIBODY

Anti-Keima-Red mAb

Code No.	Clone	Subclass	Quantity	Concentration
M182-3M	1C3	Mouse IgG1 κ	100 µL	1 mg/mL

BACKGROUND: The fluorescent proteins "Keima", which were originally cloned from the stony coral whose Japanese name is "Komon-Sango." ***CoralHue***™ mKeima-Red and dKeima-Red absorb light maximally at 440 nm and emits red light. ***CoralHue***™ dKeima570 absorbs light maximally at 440 nm and emits orange-red light. Thus ***CoralHue***™ Keima proteins exhibit an extremely large Stokes shift (130-180 nm). Because of these unique property of ***CoralHue***™ Keima proteins, it is useful for multicolor imaging.

SOURCE: This antibody was purified from hybridoma (clone 1C3) 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 protein.

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 mKeima-Red, dKeima-Red and dKeima570 (28 kDa) on Western blotting.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

INTENDED USE:

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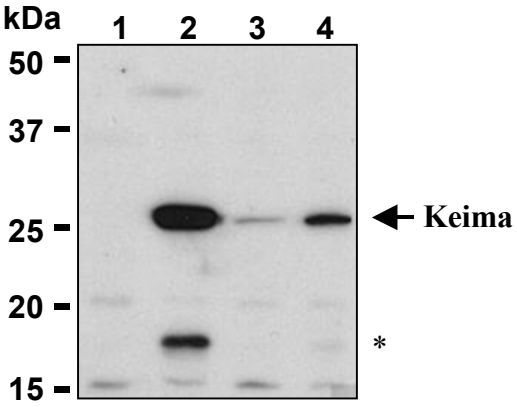
REFERENCES:

1) Kikuchi A., *et al., Biochemistry* **47**, 11573-11580 (2008)
2) Kogure, T., *et al*., *Nat. Biotechnol.* **24,** 577-581 (2006)
3) Martynov VI., *et al., J. Biol. Chem.* **278**, 46288-46292 (2003)



Western blot analysis of Keima-Red in HeLa (1), mKeima-Red transfectant (2), dKeima-Red transfectant (3), and dKeima 570 transfectant 4) using M182-3M.

**It has been reported that Orange / Red fluorescent proteins partially split into fragments, the extent of fragmentation depending on the conditions of denaturation. The 18 kDa band is is the C-terminal fragment of Keima.*

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOL:

SDS-PAGE & Western Blotting

1) Wash cells (approximately 1 x 10^7 cells) 3 times with PBS and resuspend them in 1 mL of Laemmli's sample buffer.
2) Boil the samples for 3 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 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
5) Incubate the membrane for 1 hour at room temperature with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS**. (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 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, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute.
10) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
11) Expose the membrane onto an X-ray film in a dark room for 2 minutes. Develop the film under usual settings. The conditions for exposure and development may vary.

For more information, please visit our website.
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