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

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**POLYCLONAL ANTIBODY**

# Anti-Kaede pAb

| Code No. | Quantity | Form |
|---|---|---|
| PM012M | 100 µL | Purified IgG |

**BACKGROUND:** *CoralHue*® Kaede protein emits green fluorescence that can be converted to red. The red fluorescence is comparable in intensity to the green and is stable under usual aerobic conditions. The green-to-red conversion is highly sensitive to irradiation with UV or violet light (350-410 nm). Maximal illumination results in a 2,000-fold increase in the ratio of red-to-green signal. The excitation lights used to elicit red and green fluorescence do not induce the photoconversion. This property provides a simple and powerful technique for regional optical marking.

**SOURCE:** This antibody was fractioned by salting out and gel filtration on a Sephadex G-200 column. The rabbit was immunized with the recombinant *CoralHue*® Kaede.

**FORMULATION:** 100 µL volume of 10 mM phosphate buffer containing 0.3 M NaCl and 0.09 % $NaN_3$, pH 8.0.
*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.

**STORAGE:** This antibody solution is stable for one year from the date of purchase when stored at 4°C.

**REACTIVITY:** This antibody reacts with *CoralHue*® Kaede on Western blotting.

**APPLICATIONS:**
Western blotting; 1:1,000
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested*
*It is reported that this antibody can be used in this application in the reference number 1), 3)-5) and 9)-10).
Immunocytochemistry; Not tested*
*It is reported that this antibody can be used in this application in the reference number 2) and 7).
Flow cytometry; Not tested

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

**INTENDED USE:**
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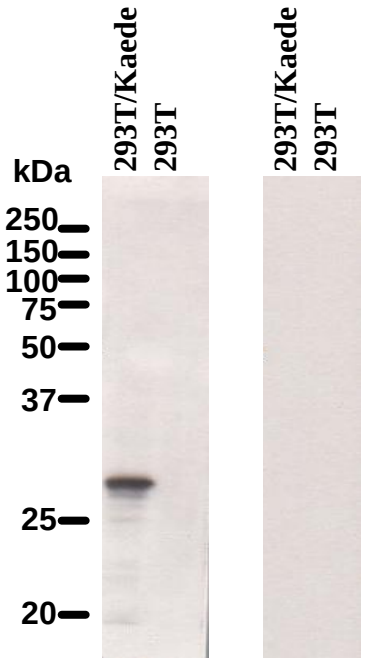


***Western blotting detection of Kaede expressed in 293T cells.***
*Left ; Immunobloted with PM012*
*Right ; Immunobloted with Normal rabbit IgG*

## PROTOCOL:

**SDS-PAGE & Western blotting**

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer [50 mM Tris-HCl (pH 7.2), 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) 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.
5) 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% methanol). See the manufacturer's manual for precise transfer procedure.
6) 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.
7) 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.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
9) Incubate the membrane with the 1:10,000 Anti-IgG (Rabbit) pAb-HRP (MBL, code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (10 minutes x 3).
11) Wipe excess buffer off the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
13) Expose to an X-ray film in a dark room for 3 minutes.
14) Develop the film as usual. The condition for exposure and development may vary.

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