

POLYCLONAL ANTIBODY

# Anti-KLF4 pAb

Code No.  
PM057

Quantity  
100 µL

Form  
Affinity Purified

**BACKGROUND:** KLF4 is a zinc-finger transcription factor expressed in the epithelium of the skin, lungs, intestine and several other organs. KLF4-KO mouse dies after birth for the abnormal formation of the skin. It is known that Klf4 cooperates with Oct3/4 and Sox2, and it is one of the factors that have been used to form iPS cells (induced pluripotent stem cells) from fibroblasts.

**SOURCE:** This antibody was purified from rabbit serum using affinity column. The rabbit was immunized with the recombinant full length of human KLF4.

**FORMULATION:** 100 µL volume of PBS containing 50% glycerol, pH 7.2. Contains no preservative.

**STORAGE:** This antibody solution is stable for one year from the date of purchase when stored at -20°C.

**REACTIVITY:** This antibody reacts with human and mouse KLF4 on Western blotting.

**APPLICATIONS:**

Western blotting: 1:1,000

Immunoprecipitation: 2 µL/300 µL of cell extract from 3 x 10<sup>6</sup> cells

Immunohistochemistry: Not tested

Immunocytochemistry: 1:200

Flow cytometry: Not tested

Detailed procedures are provided in the following **PROTOCOLS**.

**SPECIES CROSS REACTIVITY:**

Species	Human	Mouse	Rat
Cells	NCCIT, HeLa, transfectant	transfectant	Not tested
Reactivity on WB	+	+	

**INTENDED USE:**

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

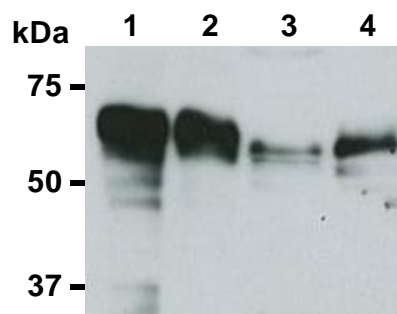
**REFERENCES:**

- 1) Takahashi, K., *et al.*, *Cell* **131**, 861-872 (2007)
- 2) Nakatake, Y., *et al.*, *Mol. Cell Biol.* **26**, 7772-7782 (2006)
- 3) Katz, J. P., *et al.*, *Development* **129**, 2619-2628 (2002)

**RELATED PRODUCTS:**

Please visit our web site <https://ruo.mbl.co.jp/>.

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



**Western blot analysis of KLF4 expression on human KLF4 transfectant (1), mouse KLF4 transfectant (2), NCCIT (3) and HeLa (4) using PM057.**

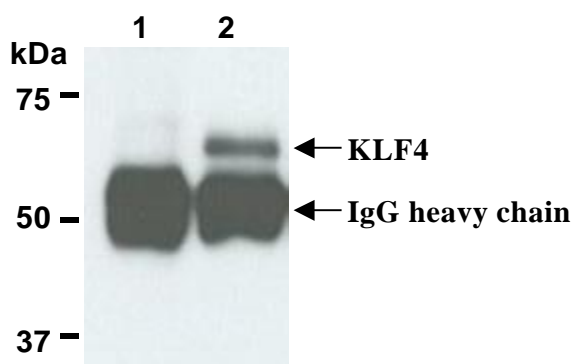
**PROTOCOLS:**

**SDS-PAGE & Western Blotting**

- 1) Wash cells (approximately 1 x 10<sup>7</sup> cells) 3 times with PBS and resuspend them in 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<sup>2</sup> 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 PBS (pH 7.2) containing 1% skimmed milk 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 HRP-conjugated anti-rabbit IgG (MBL; code no. 458) 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 10 minutes. Develop the film under usual settings. The conditions for exposure and development may vary.

(Positive controls for Western blotting; transfectant, NCCIT, HeLa)

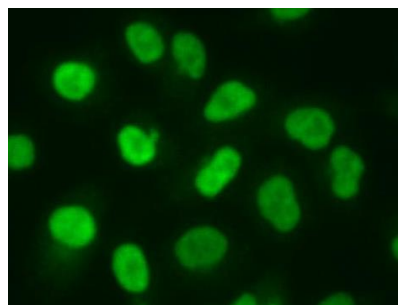


**Immunoprecipitation of KLF4 from HeLa with Normal rabbit IgG (1) or PM057 (2).** After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with PM057.

#### **Immunoprecipitation**

- 1) Wash cells (approximately  $1 \times 10^7$  cells) 3 times with PBS and resuspend them in 1 mL of cold Lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.05% NP-40) containing protease inhibitors at appropriate concentrations. Incubate it at 4°C with rotating for 30 minutes; thereafter, briefly sonicate the mixture (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.
- 3) Add primary antibody as suggested in the **APPLICATIONS** into 300 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20 µL of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
- 4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
- 5) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µL/lane for the SDS-PAGE analysis.  
(See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; HeLa)



**Immunocytochemical detection of KLF4 in HeLa with PM057.**

#### **Immunocytochemistry**

- 1) Culture the cells in the appropriate condition on a glass slide. (for example, spread  $1 \times 10^4$  cells for one slide, then incubate in a CO<sub>2</sub> incubator for one night.)
- 2) Wash the glass slide 2 times with PBS.
- 3) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde for 10 minutes at room temperature.
- 4) Wash the glass slide 3 times with PBS.
- 5) Immerse the slide in PBS containing 0.2% Triton X-100 for 10 minutes at room temperature.
- 6) Wash the glass slide 2 times with PBS.
- 7) Add the primary antibody diluted with PBS containing 2% FCS as suggested in the **APPLICATIONS** onto the cells and incubate for 1 hour at room temperature (Optimization of antibody concentration or incubation condition are recommended if necessary.)
- 8) Wash the glass slide 2 times with PBS.
- 9) Add 100 µL of 1:500 Alexa Fluor® 488 conjugated anti-rabbit IgG (Invitrogen; code no. A110374) diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
- 10) Wash the glass slide 2 times with PBS.
- 11) Wipe excess liquid off the slide but take care not to touch the cells. Never leave the cells to dry.
- 12) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; HeLa)