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

POLYCLONAL ANTIBODY

Anti-mouse Nanog

Code No.	Quantity	Form
PM058	100 µL	Affinity Purified

BACKGROUND: Embryonic stem (ES) cells are pluripotent stem cells derived from the inner cell mass of the blastocyst. Nanog containing a homeobox domain is a transcription factor expressed in ES cells, and it is required for maintenance of pluripotency in mouse epiblast and ES cells. Pluripotency of mouse ES cells can be maintained by leukemia inhibitory factor (LIF), but Nanog is capable of maintaining ES cell self-renewal independently of LIF.

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

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 mouse Nanog on Western blotting, Immunoprecipitation and Immunocytochemistry.

APPLICATIONS:

Western blotting; 1:500 for a chemiluminescence detection system
Immunoprecipitation; 2 µL/300 µL of cell extract from 3 x 10^6 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	Not Tested	P19, transfectant	Not Tested
Reactivity on WB		+	

INTENDED USE:

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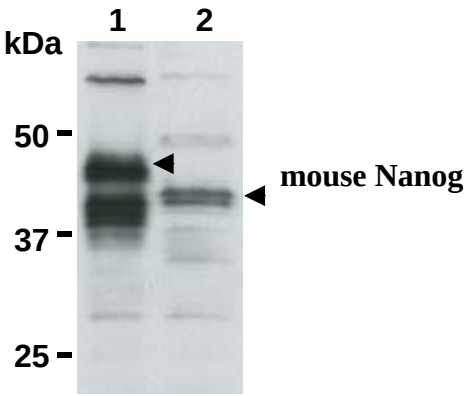
REFERENCES:

1) Junying, Yu., *et al.*, *Science* **318**, 1917-1920 (2007)
2) Mitsui, K., *et al.*, *Cell* **113**, 631-642 (2003)
3) Chambers, I., *et al.*, *Cell* **113**, 643-655 (2003)



Western blot analysis of mouse Nanog expression on His tagged mouse Nanog transfectant (1), and P19 (2) using PM058.

PROTOCOLS:

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 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 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.

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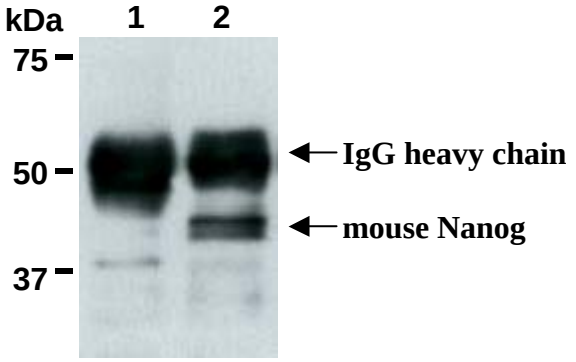
8) Wash the membrane with PBS-T (5 minutes x 3 times).
9) Wipe excess buffer off the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute.
10) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
11) Expose the membrane onto an X-ray film in a dark room for 3 minutes. Develop the film under usual settings. The conditions for exposure and development may vary.

(Positive controls for Western blotting; transfectant, P19)

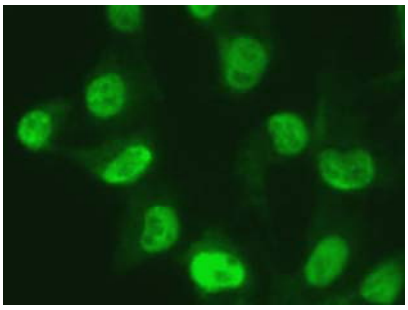


Immunoprecipitation of mouse Nanog from P19 with Normal rabbit IgG (1) or PM058 (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with PM058.*

Immunoprecipitation

1) Wash cells (approximately 1 x 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; P19)



Immunocytochemical detection of mouse Nanog in P19 with PM058.

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 1 x 10^4 cells for one slide, then incubate in a CO_2 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; P19)

RELATED PRODUCTS:

M164-3	anti-Oct3/4 (2F12)
PM048	anti-Oct3/4 (polyclonal)
PM055	anti-Lin28 (polyclonal)
PM056	anti-Sox2 (polyclonal)
PM057	anti-KLF4 (polyclonal)