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

POLYCLONAL ANTIBODY

Anti-Oct3/4 pAb

Code No.	Quantity	Form
PM048	100 µL	Affinity Purified

BACKGROUND: The POU family transcription factor Oct-3/4, termed as Oct-3 or Oct-4, encoded by Pou5f1, is expressed in totipotent/pluripotent early embryonic cells. It is also expressed in embryonic stem (ES) cells and embryonal carcinoma (EC) cells, but its expression diminishes when these cells differentiate and lose pluripotency. Oct3/4 contains three functionally characterized domains, the transcriptional activation domain of N- and C-terminal region and the POU DNA-binding domain. The POU domain binds to an octamer sequence, ATTTGCAT. Several target genes of Oct3/4, such as Sox2, contains an octamer element capable of binding Oct3/4. These sites are important for transcriptional activity. Induced pluripotent stem (iPS) cells can be generated from mouse embryonic or adult fibroblasts by induction of four factors, Oct3/4, Sox2, c-Myc, and Klf4. Oct3/4 regulates a expression of Tcl1 (T cell lymphoma break point) and Nanog and contributes to cell proliferation and stabilization of cell pluripotency. Two transcription factors Oct3/4 and Sox2 works together to control a transcriptional regulatory network that regulates the expression of other essential genes.

SOURCE: This antibody was purified from rabbit serum using affinity column. The rabbit was immunized with recombinant N-terminal of mouse Oct3/4 corresponding to 1-134 aa.

FORMULATION: 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 Oct3/4 on Western blotting, Immunoprecipitation, Immunocytochemistry and Immunohistochemistry.

APPLICATIONS:

Western blotting; 1:1,000 for chemiluminescence detection system
Immunoprecipitation; 2 µL/300 µL of cell extract from 3 x 10^6 cells
Immunohistochemistry; 1:500
Immunocytochemistry; 1:500
Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

Species	Human		Mouse		Rat	Hamster
Cells	Transfectant	HL-60, 293T, HeLa	P19 Transfectant	MEF, NIH/3T3, WR19L	Rat1	CHO
Reactivity on WB	+	-	+	-	-	-

INTENDED USE:

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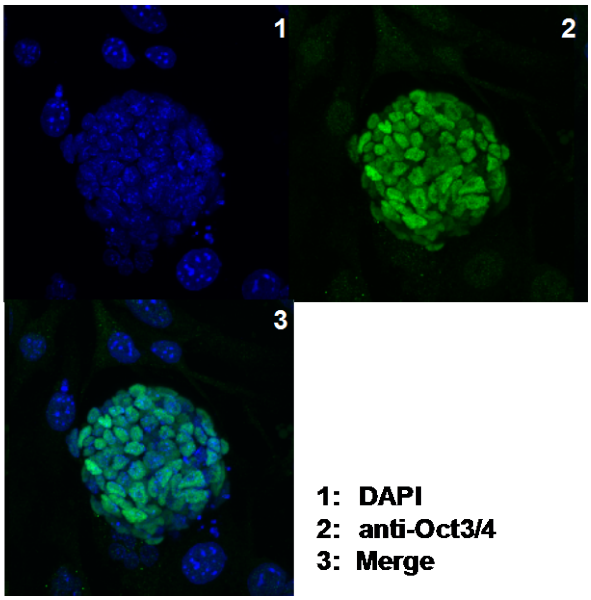
REFERENCES:

1) Boer, B., *et al.*, *Nucleic Acids Res.* **35**, 1773-1786 (2007)
2) Takahashi, K., and Yamanaka, S., *Cell* **126**, 663-676 (2006)
3) Chew, J., *et al.*, *Mol. Cell Biol.* **25**, 6031-6046 (2005)
4) Niwa, H., *et al.*, *Cell* **123**, 917-929 (2005)
5) Saijoh, Y., *et al.*, *Genes to Cells* **1**, 239-252 (1996)
6) Shimazaki, T., *et al.*, *EMBO. J.* **12**, 4489-4498 (1993)
7) Okamoto, K., *et al.*, *Cell* **60**, 461-472 (1990)

PROTOCOLS:



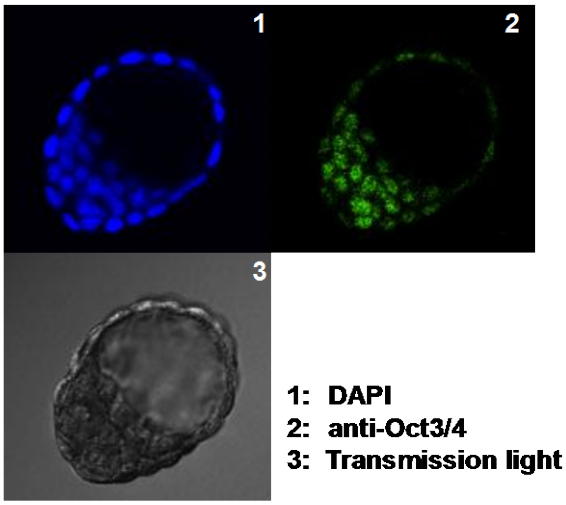
Immunocytochemical detection of Oct3/4 in mouse ES cell with PM048.
This data was kindly provided by Yoshiba and Hamada M.D., Ph.D. (Developmental Genetics Group, Graduate School of Frontier Biosciences, Osaka University)

MBL MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL http://ruo.mbl.co.jp
e-mail support@mbl.co.jp, TEL 052-238-1904

Immunocytochemistry

1) Fix the cells by immersing the glass slide in PBS containing 4% paraformaldehyde (PFA) for 20 minutes at room temperature.
2) Rinse the glass slide in 100% methanol.
3) Rinse the glass slide in PBS containing 0.1% Triton X-100.
4) Immerse the slide 5% skimmed milk in PBS containing 0.1% Triton X-100 for 1 hour at room temperature.
5) Add 30 µL of the primary antibody diluted with 5% skimmed milk, 0.1% Triton X-100 in PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 30 minutes at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
6) The glass slide was washed with PBS for 30 minutes at room temperature.
7) Add 30 µL of 1:1,000 Alexa Fluor® 488 conjugated anti-rabbit IgG (Invitrogen; code no. A11008) diluted with PBS onto the cells. Incubate for 1 hour at room temperature. Keep out light by aluminum foil.
8) The glass slide was washed with PBS for 30 minutes at room temperature.
9) Wipe excess liquid off the slide but take care not to touch the cells. Never leave the cells to dry.
10) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; mouse ES cell)



Immunohistochemical detection of Oct3/4 on frozen section of mouse embryo (E3.5) with PM048.

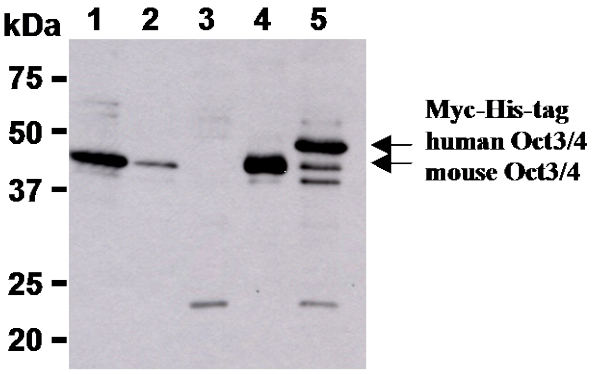
This data was kindly provided by Takaoka Ph.D. and Hamada M.D., Ph.D. (Developmental Genetics Group, Graduate School of Frontier Biosciences, Osaka University)

Immunohistochemical staining for frozen sections

For 4% paraformaldehyde fixed section

1) Wash the slide in PBS (10 minutes x 2 times).
2) Permeabilize a section with PBS containing 0.2% Triton X-100 for 10minutes.
3) Wipe gently around each section and cover tissues with blocking buffer [5% skimmed milk in PBS containing 0.1% Triton X-100] for 30 minutes to block non-specific staining. Do not wash.
4) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with blocking buffer as suggested in the **APPLICATIONS**.
5) Incubate the sections at room temperature for 1 hour.
6) Wash the slides in PBS-T [PBS containing 0.1% Triton X-100] (15 minutes x 3 times).
7) Wipe gently around each section and cover tissues with Alexa Fluor® 488 labeled anti-Rabbit IgG (Invitrogen; A11008) diluted 1:1,000 with PBS-T. Incubate for 30 minutes at room temperature.
8) Wash the slides in PBS-T (15 minutes x 2 times).
9) Wipe gently around each section and cover tissues with DAPI in PBS-T. Incubate for 5 minutes.
10) Wash the slides in PBS-T for 5 minutes.
11) Mount the slides, then put a cover slip on it.

(Positive control for Immunohistochemistry; mouse embryo)



Western blot analysis of Oct3/4 expression on P19 (1), P19 (differentiated, 2), 293T (3), mouse Oct3/4 transfectant (4) and Myc-His tag-human Oct3/4 transfectant (5) using PM048.

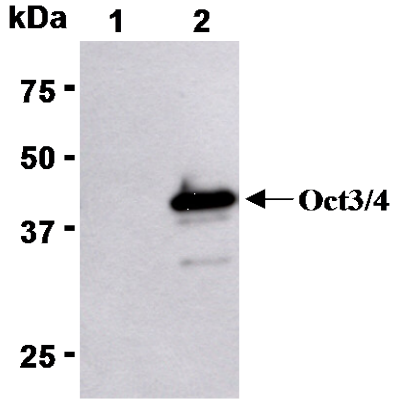
SDS-PAGE & Western Blotting

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 20 µL of the 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 5% 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 of 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.
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 control for Western blotting; P19)



Immunoprecipitation of Oct3/4 from P19 with Normal rabbit IgG (1) or PM048 (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with M164-3.

Immunoprecipitation

1) Wash cells (approximately 1 x 10^7 cells) 3 times with PBS and suspend with 10 volume of cold Lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40] 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 fresh tube.
3) Add primary antibody as suggested in the **APPLICATIONS** into 300 µL of cell extract. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C.
4) 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.
5) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
6) Resuspend the agarose in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
7) Load 10 µL of the sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
8) 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.
9) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
10) Incubate the membrane with 1 µg/mL of Anti-Oct3/4 mAb (MBL; code no. M164-3) diluted with PBS, pH 7.2 containing 1% skimmed for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
11) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
12) Incubate the membrane with 1:10,000 of 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.
13) Wash the membrane with PBS-T (5 minutes x 3 times).
14) Wipe excess buffer off the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute.
15) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
16) 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 control for Immunoprecipitation: P19)

RELATED PRODUCTS:

M164-3	Anti-Oct3/4 mAb (2F12)
D356-3	Anti-Jmjd1c (Mouse) mAb (13B)
PM055	Anti-LIN28 pAb
PM056	Anti-Sox2 pAb
PM057	Anti-KLF4 pAb
PM058	Anti-Nanog (Mouse) pAb