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

MONOCLONAL ANTIBODY

Anti-Oct3/4

Code No.	Clone	Subclass	Quantity	Concentration
M164-3	2F12	Mouse IgG2a κ	100 μg	1 mg/mL

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 hybridoma (clone 2F12) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with MRL mouse splenocyte immunized with recombinant N-terminal of mouse Oct3/4 corresponding to 1-134 aa.

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 Oct3/4 on Western blotting and Immunoprecipitation.

APPLICATIONS:

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

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

SPECIES CROSS REACTIVITY:

Species	Human		Mouse		Rat	Hamster
Cell	transfectant	293T, HeLa	P19 transfectant	MEF, NIH/3T3	PC12	CHO
Reactivity on WB	+	-	+	-	-	-

INTENDED USE:

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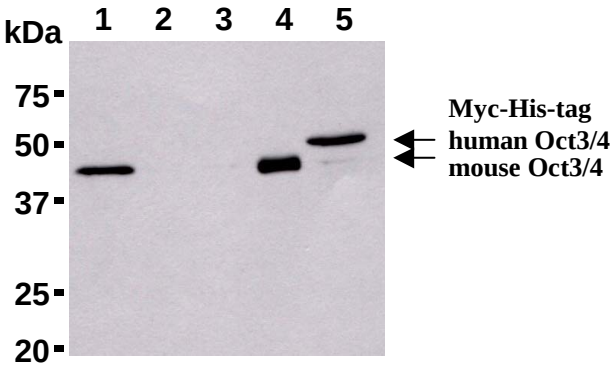
REFERENCES:

1) Boer, B., *et al.*, *Nucleic Acids Research* **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)

RELATED PRODUCTS:

PM048 anti-Oct3/4 (polyclonal)
M076-3 Mouse IgG2a isotype control (6H3)



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 M164-3.

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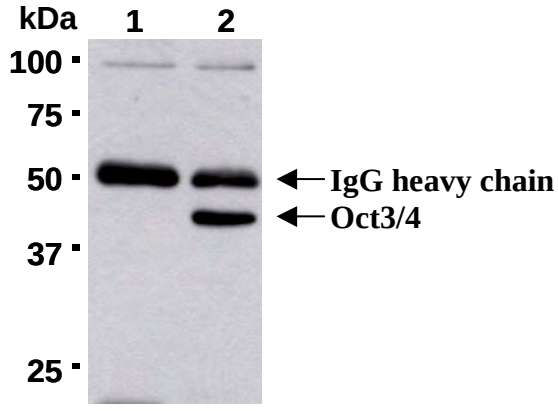
PROTOCOLS:

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 20 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 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 HRP-conjugated anti-mouse IgG (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 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 Mouse IgG2a (1) or M164-3 (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 suggest 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 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)