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

MBL

POLYCLONAL ANTIBODY

Anti-Sox2 pAb

Code No.	Quantity	Form
PM056	100 µL	Affinity Purified

BACKGROUND: Sox2, a member of the SRY-related HMG-box (SOX) family of transcription factors, is expressed in embryonic inner cell mass, epiblast, extraembryonic mesoderm. It is one of the four factors (Oct3/4, Sox2, Klf4, c-Myc) that have been used to form iPS cells (induced pluripotent stem cells) from fibroblasts. Sox2 and Oct3/4 works together to control a transcriptional regulatory network that regulates the expression of other essential genes, and plays important roles in early embryonic development.

SOURCE: This antibody was purified from rabbit serum using affinity column. The rabbit was immunized with partial recombinant mouse Sox2 protein.

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 mouse Sox2 (35 kDa) on Western blotting, Immunohistochemistry, Immunocytochemistry and Immunoprecipitation. The reactivity to human Sox2 was confirmed by Western blotting and Immunoprecipitation.

APPLICATIONS:

Western blotting; 1:1,000 for a chemiluminescence detection system
Immunoprecipitation; 2 µL/300 µL of cell extract from 3×10^6 cells
Immunohistochemistry; 1:500
Heat treatment is necessary.
Microwave oven; 500W
Frozen section: 1 mM EDTA (pH 8.0), 3 minutes
Paraffin section: 10 mM citrate buffer (pH 6.3), 20 minutes
Immunocytochemistry; 1:200
Flow cytometry; Not tested

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

INTENDED USE:
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SPECIES CROSS REACTIVITY:

Species	Human		Mouse		Rat	Chicken**
Cells	NCCIT	293T, HeLa	P19*, transfectant	NIH/3T3, WR19L	Not tested	
Reactivity on WB	+	-	+	-		

*This antibody shows weak reactivity to differentiated P19.
**The reactivity to chicken is reported in reference 2).

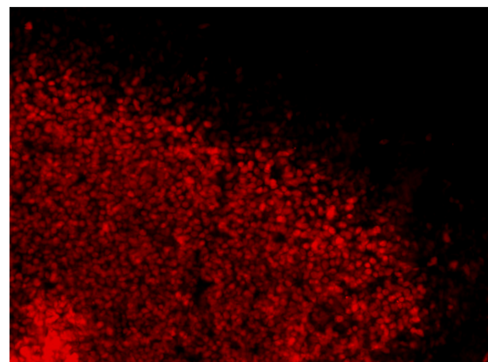
REFERENCES:

1) Suzuki, Y., *et al., Int. J. Oncol.* **42**, 161-167 (2013) [WB]
2) Gao, Y., *et al., Eur. J. Histochem.* **56**, e33 (2012) [IC]
3) Maekawa, M., *et al., Nature* **474**, 225-229 (2011)
4) Niwa, H., *Development* **134**, 635-646 (2007)
5) Yu, J., *et al., Science* **318**, 1917-1920 (2007)
6) Masui, S., *et al., Nat. Cell Biol.* **9**, 625-635 (2007)
7) Avilion, A. A., *et al., Genes & Dev.* **17**, 126-140 (2003)

This antibody is used in the reference 1)-3).

RELATED PRODUCTS:

M164-3 Anti-Oct3/4 mAb (2F12)
PM048 Anti-Oct3/4 pAb (polyclonal)
PM055 Anti-Lin28 pAb (polyclonal)



Immunohistochemical detection of Sox2 on frozen section of mouse embryo (E13) with PM056.
Fluorescence Microscope: Axiovert200
Filter set:Carl Zeiss Filter sets No.26
Lens: Plan-NEOFLUAR (Carl Zeiss), x20, NA=0.5

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

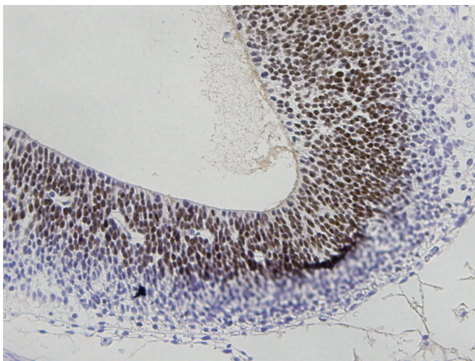
PROTOCOLS:

Immunohistochemical staining for frozen sections

For 4% paraformaldehyde fixed section

1) Wash the slide in PBS for 15 minutes.
2) Heat treatment
   Heat treatment by Microwave:
   Place the slides put on staining basket in 1 L beaker with 500 mL of 1 mM EDTA (pH 8.0). Cover the beaker with plastic wrap, then process the slides 3 minutes at 500 W with microwave oven. Let the slides cool down in the beaker at room temperature for about 40 minutes.
3) Immerse the slide in PBS containing 0.1% Tween-20 (PBS-T) for 30 minutes at room temperature.
4) Remove the slides from PBS-T, wipe gently around each section and cover tissues with blocking buffer (PBS containing 2% FCS, 0.1% Tween-20) for 5 minutes to block non-specific staining. Do not wash.
5) 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**.
6) Incubate the sections for 1 hour at room temperature.
7) Wash the slides 2 times in PBS-T for 5 minutes each.
8) Wipe gently around each section and cover tissues with 1:500 Alexa Fluor® 647 conjugated anti-rabbit IgG (Invitrogen; code no. A21245). Incubate for 30 minutes at room temperature. Wash as in step 7).
9) Wipe excess liquid off the slide but take care not to touch the section. Never leave the section to dry.
10) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunohistochemistry; Mouse embryo)

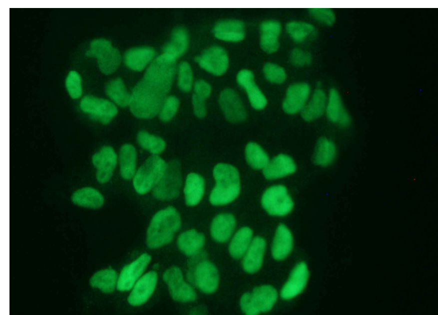


Immunohistochemical detection of Sox2 on paraffin embedded section of mouse embryo (E13) with PM056.

Immunohistochemical staining for paraffin-embedded sections

1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides 3 times in PBS for 3-5 minutes each.
4) Heat treatment
   Heat treatment by Microwave:
   Place the slides put on staining basket in 1 L beaker with 500 mL of 10 mM citrate buffer (pH 6.3). Cover the beaker with plastic wrap, then process the slides for 20 minutes at 500 W with microwave oven. Let the slides cool down in the beaker at room temperature for about 40 minutes.
5) Remove the slides from the citrate buffer (pH 6.3) and cover each section with 3% H_2O_2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 2 times in PBS for 5 minutes each.
6) Remove the slides from PBS, wipe gently around each section and cover tissues with blocking buffer (20 mM HEPES, 1% BSA, 135 mM NaCl) for 5 minutes to block non-specific staining. Do not wash.
7) 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**.
8) Incubate the sections for 1 hour at room temperature.
9) Wash the slides 3 times in PBS for 5 minutes each.
10) Wipe gently around each section and cover tissues with Histostar (Ms + Rb) (MBL; code no. 8460). Incubate for 1 hour at room temperature. Wash as in step 9).
11) Visualize by reacting for 10 minutes with DAB substrate solution (MBL; code no. 8469). *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
12) Wash the slides in water for 5 minutes.
13) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes.
14) Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each. Now ready for mounting.

(Positive control for Immunohistochemistry; mouse embryo)



Immunocytochemical detection of Sox2 in P19 with PM056.

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)

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 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 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 Anti-IgG (Rabbit) pAb-HRP (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 30 minutes 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 3 minutes. Develop the film under usual settings. The conditions for exposure and development may vary.

(Positive controls for Western blotting; NCCIT and HeLa)

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 fresh tube.
3) Add primary antibody as suggested in the **APPLICATIONS** into 200 µL of the supernatant. Mix well and incubate with gentle agitation for 60-120 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 20 µL/lane for the SDS-PAGE analysis.
   (See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation: HeLa)