

POLYCLONAL ANTIBODY

Anti-Sox2 pAb

Code No.
PM056

Quantity
100 µL

Form
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 x 10⁶ 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:

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

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).

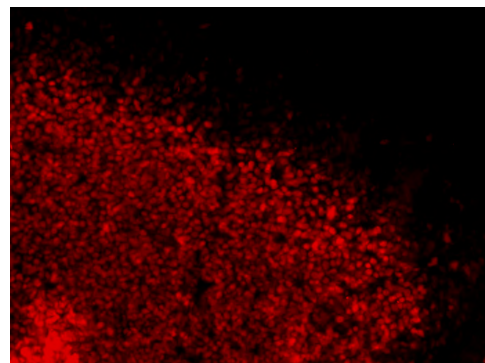
REFERENCES:

- 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

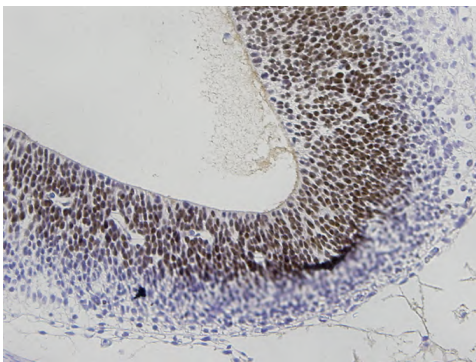
Lens: Plan-NEOFLUAR (Carl Zeiss), x20, NA=0.5

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

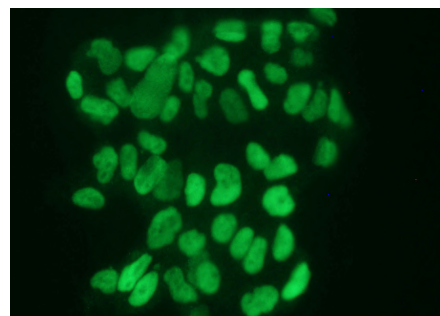
- 1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
- 2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
- 3) Wash the slides 3 times in PBS for 3-5 minutes each.
- 4) Heat treatment

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% H₂O₂ 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 1×10^4 cells for one slide, then incubate in a CO₂ 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 μ L 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 1×10^7 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 μ L 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/cm² 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 1×10^7 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 μ L of the supernatant. Mix well and incubate with gentle agitation for 60-120 minutes at 4°C.
- 4) Add 20 μ L 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 μ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 20 μ L/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting.**)

(Positive control for Immunoprecipitation: HeLa)