Anti-SOX9 Antibody [PSH13-59] - BSA and Azide free

HA751478

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	IHC-Fr, IHC-P, WB, IF-Cell, IP, FC, ChIP
Molecular Wt:	Predicted band size: 56 kDa
Clone number:	PSH13-59

Description: SOX-9 recognizes the sequence CCTTGAG along with other members of the HMG-box class DNA-binding proteins. It is expressed by proliferating but not hypertrophic chondrocytes that is essential for differentiation of precursor cells into chondrocytes and, with steroidogenic factor 1, regulates transcription of the anti-Müllerian hormone (AMH) gene. SOX-9 also plays a pivotal role in male sexual development; by working with Sf1, SOX-9 can produce AMH in Sertoli cells to inhibit the creation of a female reproductive system. It also interacts with a few other genes to promote the development of male sexual organs. The process starts when the transcription factor Testis determining factor (encoded by the sex-determining region SRY of the Y chromosome) activates SOX-9 activity by binding to an enhancer sequence upstream of the gene. Next, Sox9 activates FGF9 and forms feedforward loops with FGF9[and PGD2. These loops are important for producing SOX-9; without these loops, SOX-9 would run out and the development of a female would almost certainly ensue. Activation of FGF9 by SOX-9 starts vital processes in male development, such as the creation of testis cords and the multiplication of Sertoli cells.

Immunogen:	Recombinant protein within mouse SOX9 aa 1-450.
Positive control:	Human colon tissue, mouse brain tissue, mouse colon tissue, rat brain tissue, rat colon tissue, NIH/3T3 cell lysate, PANC-1 cell lysate, SW480 cell lysate, MDA-MB-231 cell lysate, NIH/3T3.
Subcellular location:	Nucleus.
Database links:	SwissProt: P48436 Human | Q04887 Mouse | F1LYL9 Rat

Recommended Dilutions:

IHC-Fr	1:500
IHC-P	1:1,000
WB	1:5,000
IF-Cell	1:2,000-1:5,000
IP	1-2μg/sample
FC	1:1,000
ChIP	Use 5 μg for 25 μg of chromatin.

Storage Buffer:	PBS (pH7.4).
Storage Instruction:	Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.
Purity:	Protein A affinity purified.

Images

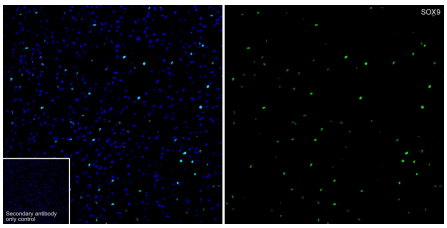

Fig1: Application: IHC-Fr

Species: Mouse

Site: brain

Sample: Frozen section

Antibody concentration: 1/500

Antigen retrieval: Not required

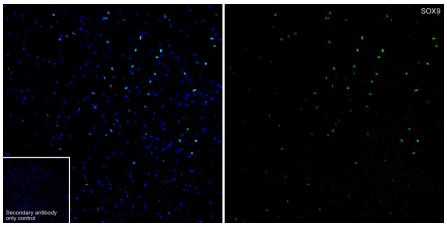

Fig2: Application: IHC-Fr

Species: Rat

Site: brain

Sample: Frozen section

Antibody concentration: 1/500

Antigen retrieval: Not required

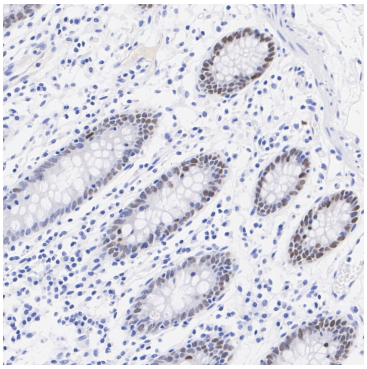

Fig3: Immunohistochemical analysis of paraffin-embedded human colon tissue with Rabbit anti-SOX9 antibody (HA751478) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA751478) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

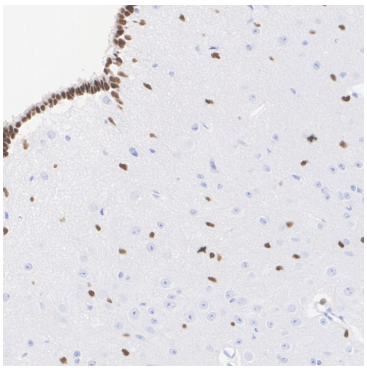

Fig4: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-SOX9 antibody (HA751478) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA751478) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

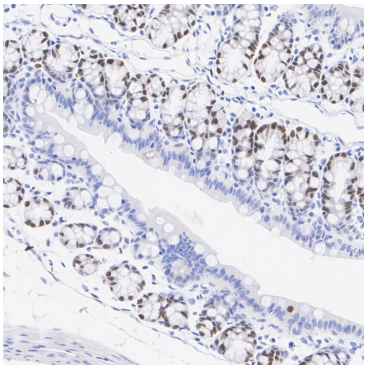

Fig5: Immunohistochemical analysis of paraffin-embedded mouse colon tissue with Rabbit anti-SOX9 antibody (HA751478) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA751478) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

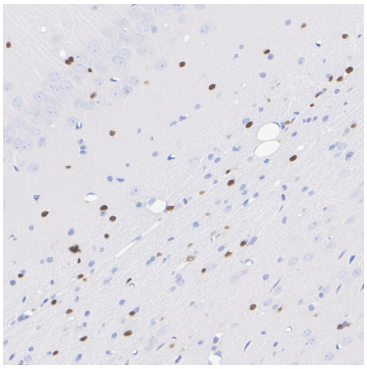

Fig6: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-SOX9 antibody (HA751478) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA751478) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

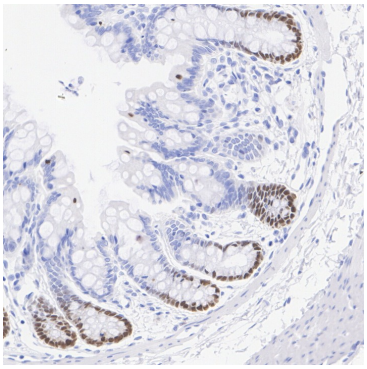

Fig7: Immunohistochemical analysis of paraffin-embedded rat colon tissue with Rabbit anti-SOX9 antibody (HA751478) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA751478) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

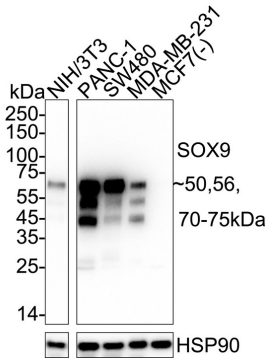

Fig8: Western blot analysis of SOX9 on different lysates with Rabbit anti-SOX9 antibody (HA751478) at 1/5,000 dilution.

Lane 1: NIH/3T3 cell lysate
Lane 2: PANC-1 cell lysate
Lane 3: SW480 cell lysate
Lane 4: MDA-MB-231 cell lysate
Lane 5: MCF7 cell lysate (negative)

Lysates/proteins at 20 µg/Lane.

Predicted band size: 56 kDa
Observed band size: 50-70 kDa

Exposure time: 18 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA751478) at 1/5,000 dilution was used in primary antibody dilution (K1803) at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

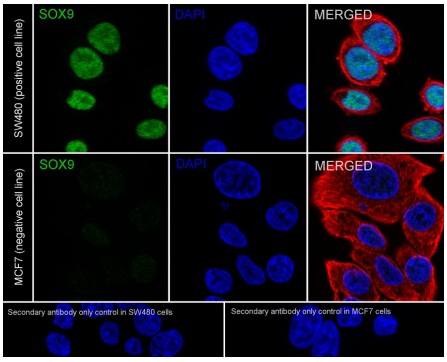

Fig9: Immunocytochemistry analysis of SW480 cells (positive) and MCF7 cells (negative) labeling SOX9 with Rabbit anti-SOX9 antibody (HA751478) at 1/5,000 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-SOX9 antibody (HA751478) at 1/5,000 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

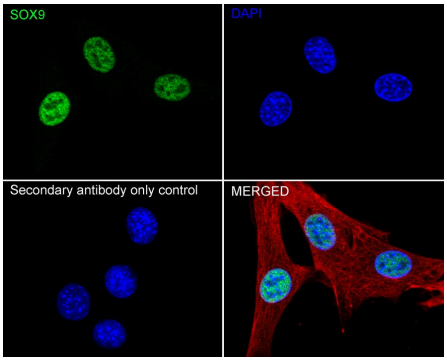

Fig10: Immunocytochemistry analysis of NIH/3T3 cells labeling SOX9 with Rabbit anti-SOX9 antibody (HA751478) at 1/2,000 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-SOX9 antibody (HA751478) at 1/2,000 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

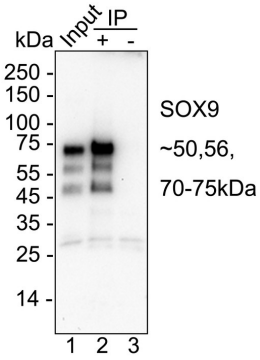

Fig11: SOX9 was immunoprecipitated from 0.2 mg MDA-MB-231 cell lysate with HA751478 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA751478 at 1/5,000 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: MDA-MB-231 cell lysate (input)
Lane 2: HA751478 IP in MDA-MB-231 cell lysate
Lane 3: Rabbit IgG instead of HA751478 in MDA-MB-231 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 46 seconds; ECL: K1801

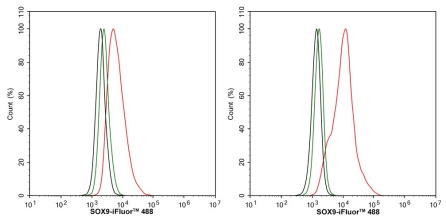

Fig12: Flow cytometric analysis of MCF7 (left, negative) and SW480 (right, positive) cells labeling SOX9.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA751478, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

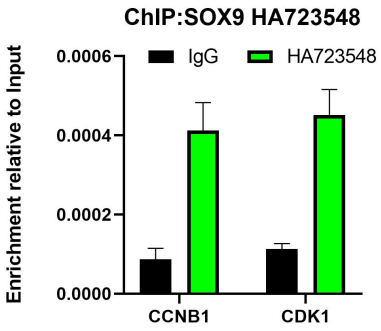

Fig13: Chromatin immunoprecipitations were performed with cross-linked chromatin from SW480 cells with SOX9 (HA751478) or Normal Rabbit IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated primers. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

Background References

1. Aggarwal S et al. SOX9 switch links regeneration to fibrosis at the single-cell level in mammalian kidneys. Science. 2024 Feb
2. Liu Y et al. Yap-Sox9 signaling determines hepatocyte plasticity and lineage-specific hepatocarcinogenesis. J Hepatol. 2022 Mar