Anti-SOX9 Antibody [SN74-09] - BSA and Azide free

HA750262

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Cynomolgus monkey, Pig
Applications:	WB, IF-Cell, IHC-P, IP, FC, CUT&Tag-seq
Molecular Wt:	Predicted band size: 56 kDa
Clone number:	SN74-09

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 human SOX9 aa 140-340.
Positive control:	SW480 cell lysate, MDA-MB-231 cell lysate, PANC-1 cell lysate, NIH/3T3 cell lysate, MDA-MB-231, human tonsil tissue, human breast carcinoma tissue, mouse liver tissue, human colon tissue, human thyroid tissue, human breast tissue, human small intestine tissue, human prostate carcinoma tissue, human stomach carcinoma tissue, human lung carcinoma tissue, rat colon tissue.
Subcellular location:	Nucleus.
Database links:	SwissProt: P48436 Human | Q04887 Mouse | F1LYL9 Rat
Recommended Dilutions:	
WB	1:10,000
IF-Cell	1:500
IHC-P	1:200-1:1,000
FC	1:2,000
IP	Use at an assay dependent concentration.
mIHC	1:1,500
Storage Buffer:	PBS (pH7.4).
Storage Instruction:	Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.
Purity:	Protein A affinity purified.

Images

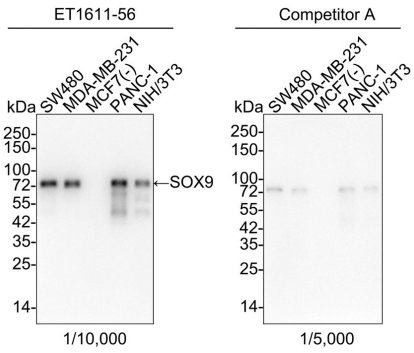

Fig1: Western blot analysis of SOX9 on different lysates with Rabbit anti-SOX9 antibody (HA750262) at 1/10,000 dilution and competitor's antibody at 1/5,000 dilution.

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

Lysates/proteins at 10 µg/Lane.

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

Exposure time: 1 minute 10 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 (HA750262) at 1/10,000 dilution and competitor's antibody at 1/5,000 dilution were used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

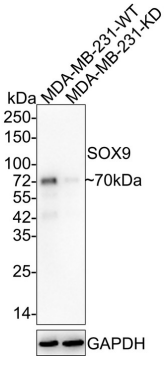

Fig2: Western blot analysis of SOX9 on different lysates with Rabbit anti-SOX9 antibody (HA750262) at 1/10,000 dilution.

Lane 1: MDA-MB-231-si NT cell lysate
Lane 2: MDA-MB-231-si SOX9 cell lysate

Lysates/proteins at 10 µg/Lane.

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

Exposure time: 1 minute 50 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 (HA750262) at 1/10,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

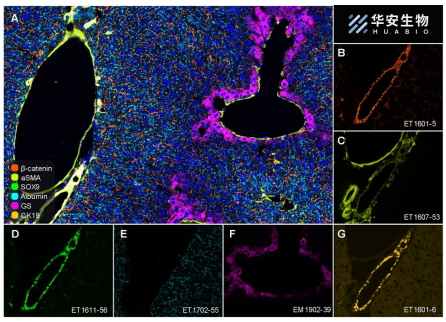

Fig3: Fluorescence multiplex immunohistochemical analysis of mouse liver (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-β-catenin (ET1601-5, Tangerine), anti-αSMA (ET1607-53, Yellow), anti-SOX9 (HA750262, Green), anti-Albumin (ET1702-55, Cyan) anti-GS (EM1902-39, Magenta) and anti-CK19 (ET1601-6, Orange) on mouse liver. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in six rounds of staining: in the order of ET1601-5 (1/2,000 dilution), ET1607-53 (1/3,000 dilution), ET1611-56 (1/1,500 dilution), ET1702-55 (1/3,000 dilution), EM1902-39 (1/2,000 dilution) and ET1601-6 (1/3,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

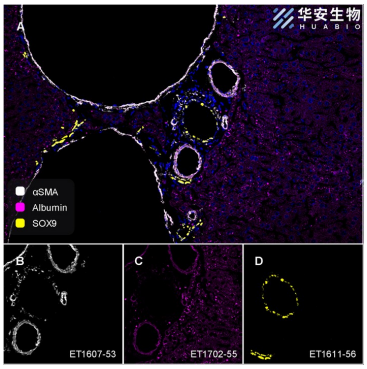

Fig4: Fluorescence multiplex immunohistochemical analysis of mouse liver (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-Albumin (ET1702-55, Violet), anti-SOX9 (HA750262, Yellow) and anti-αSMA (ET1607-53, White) on liver. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH900207). The section was incubated in three rounds of staining: in the order of ET1702-55 (1/3,000 dilution), ET1611-56 (1/1,500 dilution) and ET1607-53 (1/3,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Zeiss Observer 7 Inverted Fluorescence Microscope.

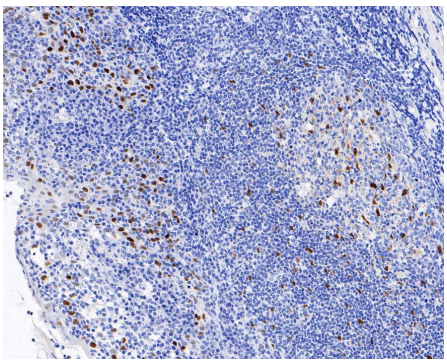

Fig5: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-SOX9 antibody (HA750262) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (HA750262) 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.

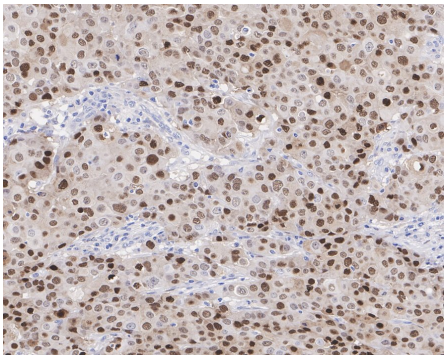

Fig6: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue with Rabbit anti-SOX9 antibody (HA750262) at 1/1,000 dilution.

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 (HA750262) 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.

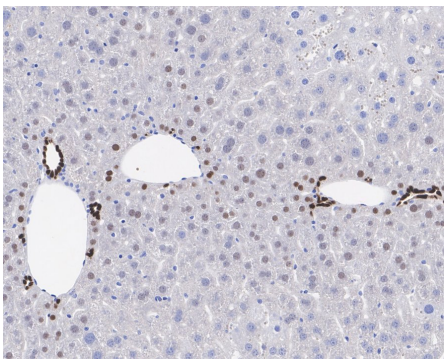

Fig7: Immunohistochemical analysis of paraffin-embedded mouse liver tissue with Rabbit anti-SOX9 antibody (HA750262) at 1/1,000 dilution.

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 (HA750262) 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.

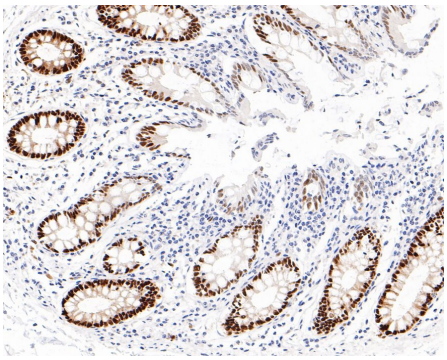

Fig8: Immunohistochemical analysis of paraffin-embedded human colon tissue with Rabbit anti-SOX9 antibody (HA750262) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (HA750262) 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.

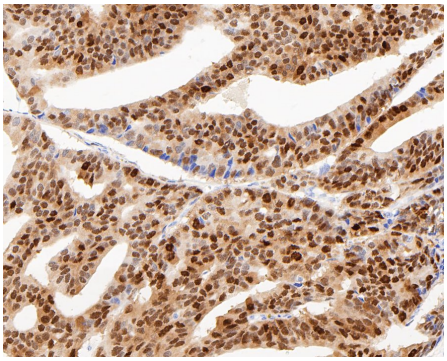

Fig9: Immunohistochemical analysis of paraffin-embedded human prostate carcinoma tissue with Rabbit anti-SOX9 antibody (HA750262) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (HA750262) at 1/200 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.

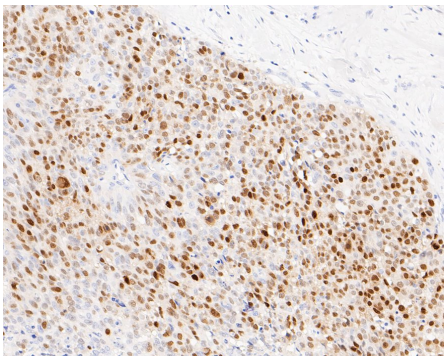

Fig10: Immunohistochemical analysis of paraffin-embedded human lung carcinoma tissue with Rabbit anti-SOX9 antibody (HA750262) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (HA750262) at 1/200 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.

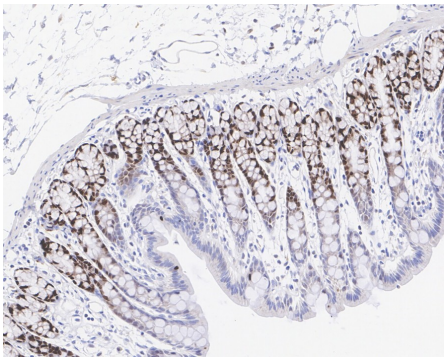

Fig11: Immunohistochemical analysis of paraffin-embedded rat colon tissue with Rabbit anti-SOX9 antibody (HA750262) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (HA750262) 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.

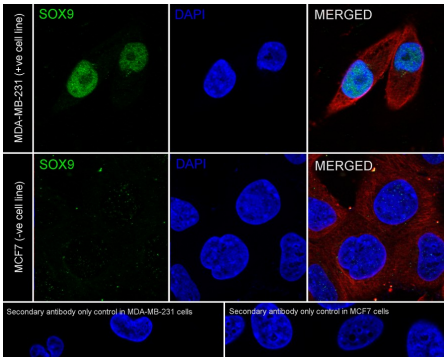

Fig12: Immunocytochemistry analysis of MDA-MB-231 (positive) and MCF7 (negative) labeling SOX9 with Rabbit anti-SOX9 antibody (HA750262) at 1/500 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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 (HA750262) at 1/500 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 (M1305-2, 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.

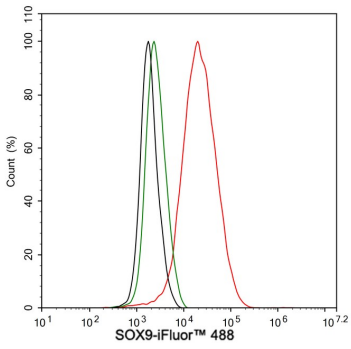

Fig13: Flow cytometric analysis of MDA-MB-231 cells labeling SOX9.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750262, 1/2,000) (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).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

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