Anti-SOX2 Antibody [PO00-28]

HA721155

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	IHC-P, WB, IF-Cell, ChIP, IHC-Fr, IF-Tissue
Molecular Wt:	Predicted band size: 34 kDa
Clone number:	PO00-28

Description: The differentiation of seminomas from non-seminomatous germ cell tumors can be challenging, especially, if small biopsy specimens, necrotic tumors and metastatic tumors with artifacts are encountered. A subset of germ cell tumors may require immunohistochemistry (IHC) for classification owing to unusual morphologic features, such as diffuse growth of clear cells, and tumors with glandular and/or microcytic patterns.1 In the mixed germ cell tumor, one component is often intermingled intimately with others such as embryonal carcinoma versus yolk sac tumor, can be overlooked. IHC will identify such an area and allow for the identification of each component of the mixed tumor more accurately and documenting them in the pathology report is recommended by WHO. Current IHC studies have shown the combination of CD30/CD117 staining plays a good role in distinguishing between embryonal carcinoma and yolk sac tumor. However, a subset of tumors may not be distinguished by this combination. Also, the characteristic membranous pattern by antibodies to CD30 and CD117 for the interpretation of the diagnosis may not be evident in limited biopsy specimens. In this respect, transcription factors, such as SOX-2, are easier to interpret due to their distinct nuclear reaction. SOX-2 has been reported as a diagnostic marker for embryonal carcinoma. SOX-2 was expressed in intratubular embryonal carcinoma, pure embryonal carcinoma and in the embryonal carcinoma component of mixed germ cell tumor in all cases. But, SOX-2 expression has not been found in seminoma, yolk sac tumor, and choriocarcinoma in almost all cases.

Immunogen: Recombinant protein within human SOX2 aa 1-317.

Positive control: NCCIT cell lysates, F9 cell lysates, NCCIT, F9, human cervical carcinoma tissue, human glioma tissue, human esophagus tissue, human tonsil tissue, human lung carcinoma tissue, human trachea tissue, mouse brain tissue, mouse lung tissue, rat brain tissue, rat hippocampus tissue.

Subcellular location: Nucleus.

Database links: SwissProt: P48431 Human | P48432 Mouse
Entrez Gene: 499593 Rat

Recommended Dilutions:

IHC-P	1:1,000-1:4,000
WB	1:1,000
IF-Cell	1:100-1:500
ChIP	Use 0.5~2 μg for 25 μg of chromatin.
IHC-Fr	1:500
IF-Tissue	1:500

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

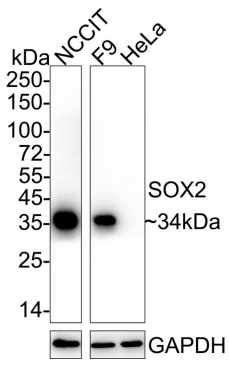

Fig1: Western blot analysis of SOX2 on different lysates with Rabbit anti-SOX2 antibody (HA721155) at 1/2,000 dilution.

Lane 1: NCCIT cell lysate
Lane 2: F9 cell lysate
Lane 3: HeLa cell lysate (negative)

Predicted band size: 34 kDa
Observed band size: 34 kDa

Exposure time: 15 seconds;

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 (HA721155) at 1/2,000 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.

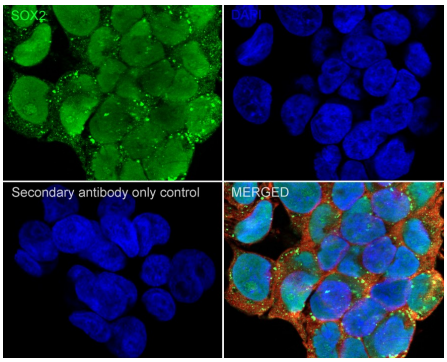

Fig2: Immunocytochemistry analysis of NCCIT cells labeling SOX2 with Rabbit anti-SOX2 antibody (HA721155) at 1/100 dilution.

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-SOX2 antibody (HA721155) at 1/100 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.

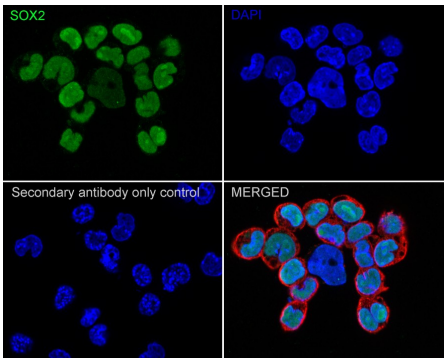

Fig3: Immunocytochemistry analysis of F9 cells labeling SOX2 with Rabbit anti-SOX2 antibody (HA721155) at 1/500 dilution.

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-SOX2 antibody (HA721155) 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.

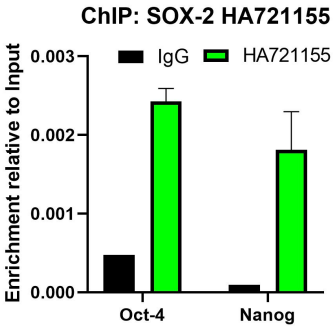

Fig4: Chromatin immunoprecipitations were performed with cross-linked chromatin from NCCIT cells and either SOX2 (HA721155) or Normal Rabbit IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated primers. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

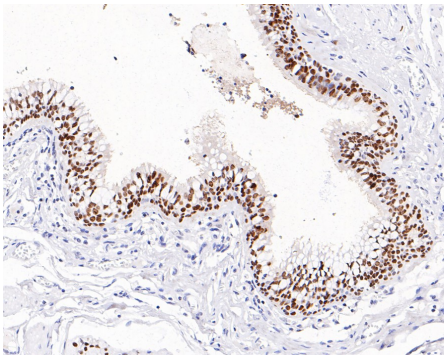

Fig5: Immunohistochemical analysis of paraffin-embedded human trachea tissue with Rabbit anti-SOX2 antibody (HA721155) at 1/1,000 dilution.

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 (HA721155) 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.

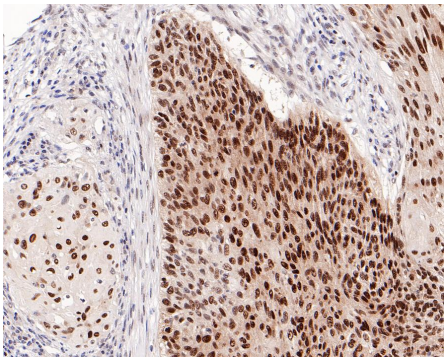

Fig6: Immunohistochemical analysis of paraffin-embedded human cervical carcinoma tissue with Rabbit anti-SOX2 antibody (HA721155) at 1/1,000 dilution.

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 (HA721155) 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.

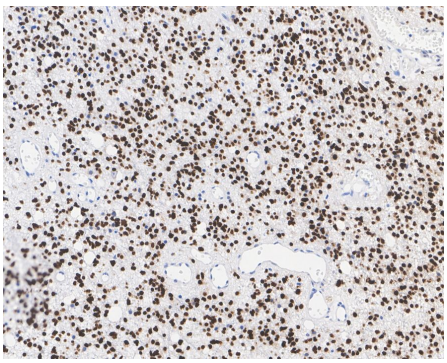

Fig7: Immunohistochemical analysis of paraffin-embedded human glioma tissue with Rabbit anti-SOX2 antibody (HA721155) at 1/4,000 dilution.

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 (HA721155) at 1/4,000 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.

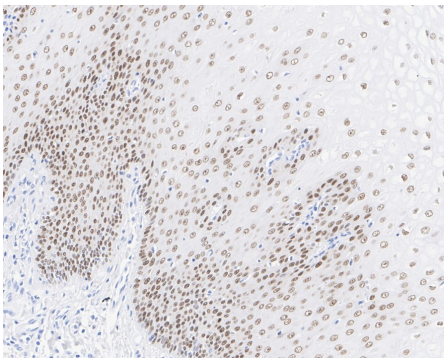

Fig8: Immunohistochemical analysis of paraffin-embedded human esophagus tissue with Rabbit anti-SOX2 antibody (HA721155) at 1/4,000 dilution.

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 (HA721155) at 1/4,000 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.

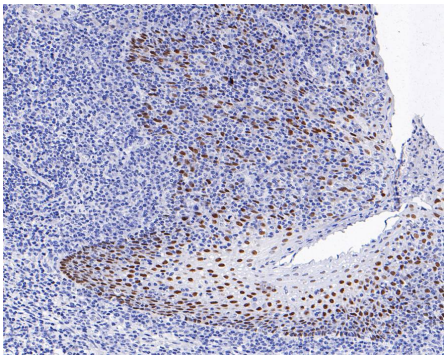

Fig9: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-SOX2 antibody (HA721155) at 1/1,000 dilution.

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 (HA721155) 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.

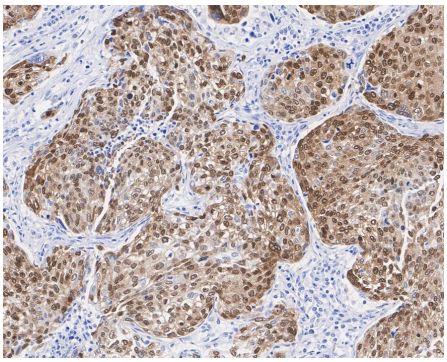

Fig10: Immunohistochemical analysis of paraffin-embedded human lung cancer tissue with Rabbit anti-SOX2 antibody (HA721155) at 1/4,000 dilution.

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 (HA721155) at 1/4,000 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.

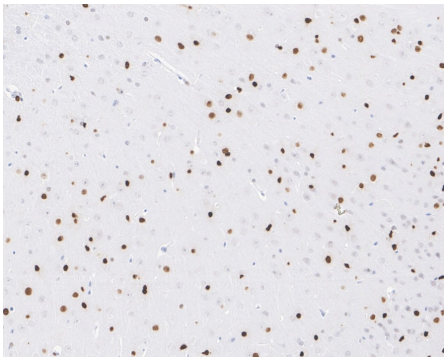

Fig11: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-SOX2 antibody (HA721155) at 1/4,000 dilution.

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 (HA721155) at 1/4,000 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.

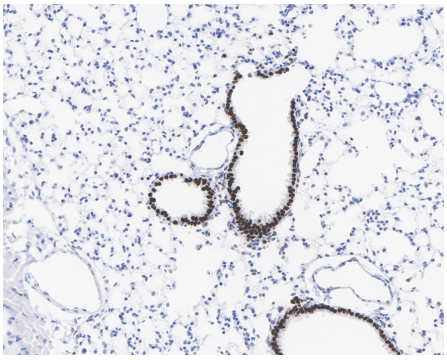

Fig12: Immunohistochemical analysis of paraffin-embedded mouse lung tissue with Rabbit anti-SOX2 antibody (HA721155) at 1/1,000 dilution.

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 (HA721155) 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.

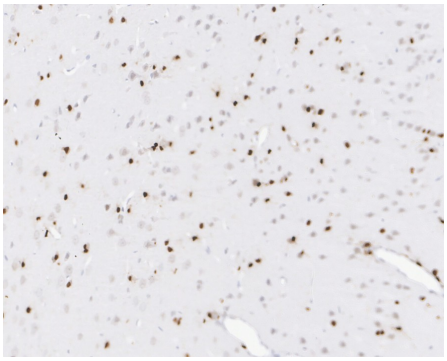

Fig13: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-SOX2 antibody (HA721155) at 1/4,000 dilution.

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 (HA721155) at 1/4,000 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.

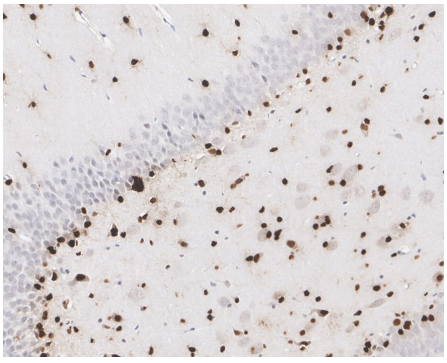

Fig14: Immunohistochemical analysis of paraffin-embedded rat hippocampus tissue with Rabbit anti-SOX2 antibody (HA721155) at 1/1,000 dilution.

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 (HA721155) 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.

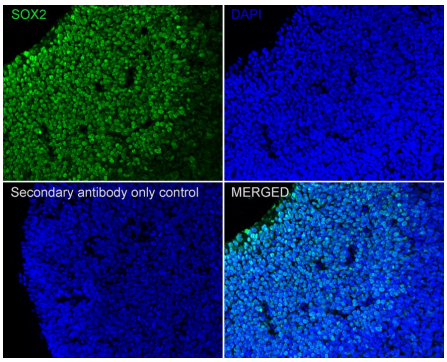

Fig15: Immunofluorescence analysis of frozen E14.5 mouse embryonic brain tissue with Rabbit anti-SOX2 antibody (HA721155) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA721155, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

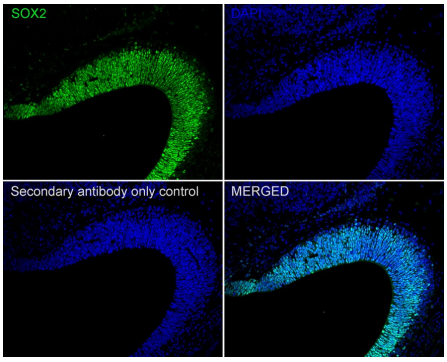

Fig16: Immunofluorescence analysis of paraffin-embedded E14.5 mouse embryonic brain tissue labeling SOX2 with Rabbit anti-SOX2 antibody (HA721155) at 1/500 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA721155, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

Background References

1. Novak D. et. al. SOX2 in development and cancer biology. Semin Cancer Biol. 2020 Dec
2. Porter L. et. al. SOX2 and squamous cancers. Semin Cancer Biol. 2020 Dec