Anti-SOX10 Antibody [PDH0-03]

HA721240

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell, IF-Tissue, FC, IHC-Fr, IP
Molecular Wt:	Predicted band size: 50 kDa
Clone number:	PDH0-03

Description: SOX10 is a neural crest transcription factor crucial for specification, maturation, and maintenance of Schwann cells and melanocytes. SOX10 is involved in the generation of myelin through interaction with OLIG1. In melanocytic cells the SOX10 gene expression is regulated by microphtalmia transcription factor. The SOX10 nuclear protein is widely expressed in glial cells, Schwann cells, and myoepithelial cells (salivary, bronchial, and mammary glands). SOX10 is expressed in virtually all cases of naevus and malignant melanoma (including 97-100% of desmoplastic and spindle cell melanomas), schwannoma, neurofibroma and granular cell tumour. SOX10 is expressed in the majority of cases of oligodendroglioma, astrocytoma and glioblastoma (few studies), desmoplastic/triple-negative breast carcinoma, acinic cell carcinoma, adenoid cystic carcinoma, epithelial-myoepithelial and myoepithelial carcinoma, and pleomorphic adenoma component of salivary gland adenocarcinoma. SOX10 is expressed in about half of cases of malignant nerve sheath tumour, and clear cell sarcoma (tendons and aponeuroses). Rare cases of luminal type breast ductal carcinoma and synovial sarcoma have shown SOX10 positivity. Apart from the above mentioned, epithelial and mesenchymal tumours are uniformly SOX10 negative. In paragangliomas/phaeochromocytomas and epithelial neuroendocrine tumours, SOX10 is only expressed in sustentacular cells but not in tumour cells. SOX10 is more specific than S100 protein in the detection of melanocytic and schwannian neoplasms, and has in some studies shown more sensitive. Skin and colon/appendix are recommended as positive and negative tissue controls for SOX10. In skin, moderate to strong nuclear staining reaction in virtually all melanocytes must be seen. The vast majority of myoepithelial cells lining sweat glands must show an at least moderate nuclear staining reaction. In colon/appendix, virtually all Schwann cells must display an as strong as possible nuclear staining reaction without any staining reaction of epithelial cells and smooth muscle cells. At present, and as specified in previous assessments, no reliable tissue component with consistent low level expression of SOX10 has been identified, monitoring the overall analytical sensitivity of an assay. Due to this issue both skin and colon/appendix are needed as tissue controls for SOX10.

Immunogen:	Synthetic peptide within C terminal human SOX10.
Positive control:	Mouse brain tissue, mouse cerebellum tissue, rat cerebellum tissue, human breast tissue, human malignant melanoma tissue, A735 cell lysate, B16-F1 cell lysate, C6 cell lysate, A375, B16F1.
Subcellular location:	Cytoplasm, Membrane, Mitochondrion, Mitochondrion outer membrane, Nucleus
Database links:	SwissProt: P56693 Human | Q04888 Mouse | O55170 Rat

Recommended Dilutions:

WB	1:1,000-1:2,000
IHC-P	1:1,000-1:3,000
IF-Cell	1:50
IF-Tissue	1:50
FC	1:500-1:1,000
IHC-Fr	1:500
IP	1-2μg/sample

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

Images

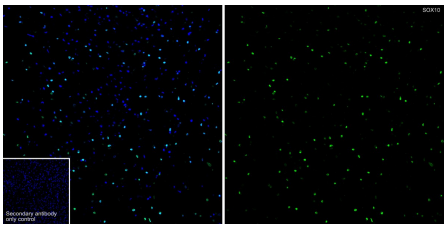

Fig1: Immunofluorescence analysis of frozen mouse brain tissue with Rabbit anti-SOX10 antibody (HA721240) at 1/500 dilution.

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 (HA721240, 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).

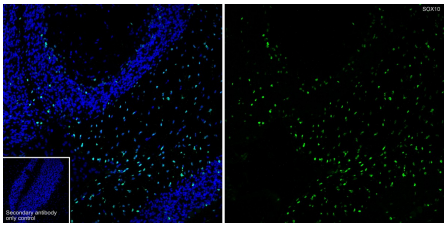

Fig2: Immunofluorescence analysis of frozen rat cerebellum tissue with Rabbit anti-SOX10 antibody (HA721240) at 1/500 dilution.

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 (HA721240, 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).

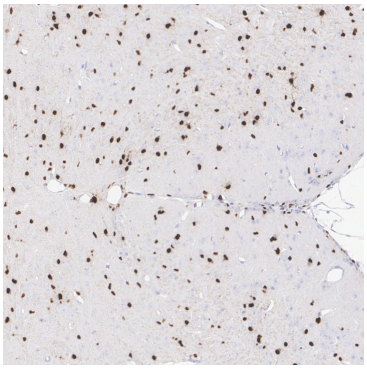

Fig3: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-SOX10 antibody (HA721240) at 1/1,000 dilution.

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 (HA721240) 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.

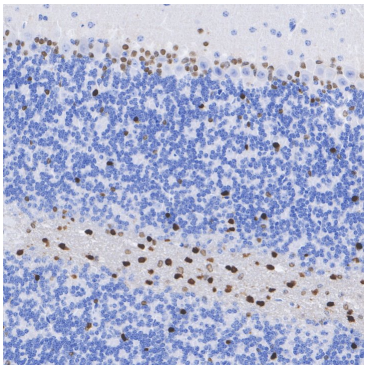

Fig4: Immunohistochemical analysis of paraffin-embedded mouse cerebellum tissue with Rabbit anti-SOX10 antibody (HA721240) at 1/1,000 dilution.

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 (HA721240) 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.

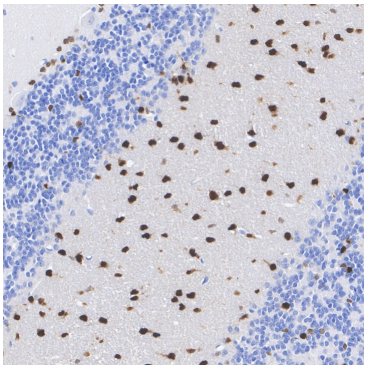

Fig5: Immunohistochemical analysis of paraffin-embedded rat cerebellum tissue with Rabbit anti-SOX10 antibody (HA721240) at 1/1,000 dilution.

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 (HA721240) 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.

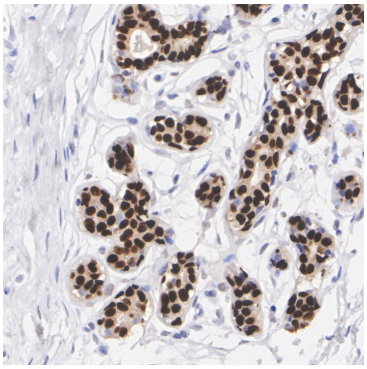

Fig6: Immunohistochemical analysis of paraffin-embedded human breast tissue with Rabbit anti-SOX10 antibody (HA721240) at 1/1,000 dilution.

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 (HA721240) 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.

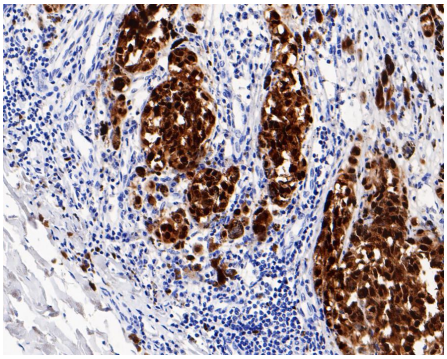

Fig7: Immunohistochemical analysis of paraffin-embedded human malignant melanoma tissue with Rabbit anti-SOX10 antibody (HA721240) at 1/3,000 dilution.

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 (HA721240) at 1/3,000 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.

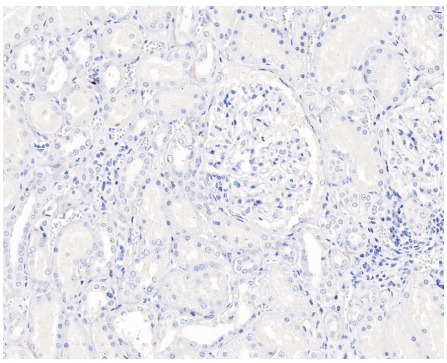

Fig8: Immunohistochemical analysis of paraffin-embedded human kidney tissue (Negative control) with Rabbit anti-SOX10 antibody (HA721240) at 1/3,000 dilution.

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 (HA721240) at 1/3,000 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.

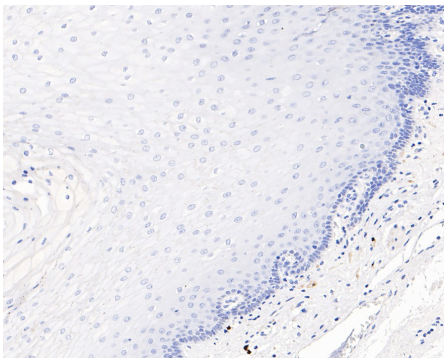

Fig9: Immunohistochemical analysis of paraffin-embedded human esophagus tissue (Negative control) with Rabbit anti-SOX10 antibody (HA721240) at 1/3,000 dilution.

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 (HA721240) at 1/3,000 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.

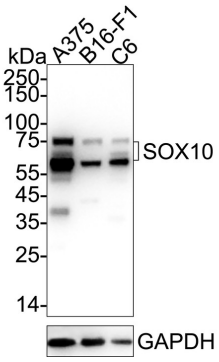

Fig10: Western blot analysis of SOX10 on different lysates with Rabbit anti-SOX10 antibody (HA721240) at 1/1,000 dilution.

Lane 1: A735 cell lysate
Lane 2: B16-F1 cell lysate
Lane 3: C6 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 50 kDa
Observed band size: 60-75 kDa

Exposure time: 4 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 (HA721240) at 1/1,000 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.

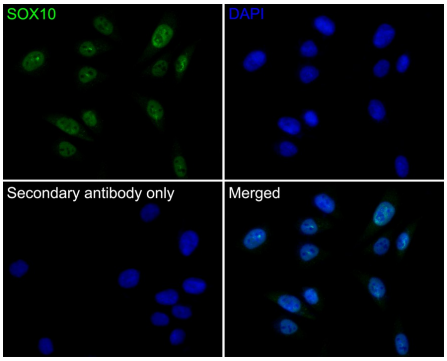

Fig11: Immunocytochemistry analysis of A375 cells labeling SOX10 with Rabbit anti-SOX10 antibody (HA721240) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-SOX10 antibody (HA721240) at 1/50 dilution in 2% negative goat serum 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.

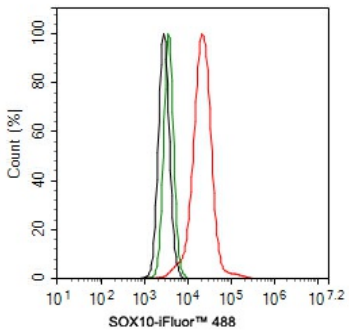

Fig12: Flow cytometric analysis of A375 cells labeling SOX10.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721240, 1ug/ml) (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).

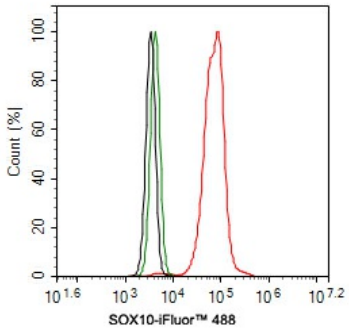

Fig13: Flow cytometric analysis of B16F1 cells labeling SOX10.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721240, 1ug/ml) (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).

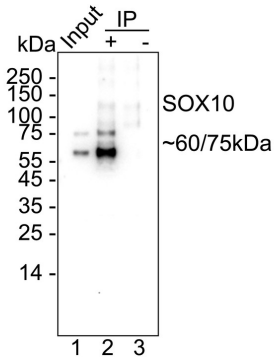

Fig14: SOX10 was immunoprecipitated from 0.2 mg A375 cell lysate with HA721240 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using HA721240 at 1/2,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: A375 cell lysate (input)
Lane 2: HA721240 IP in A375 cell lysate
Lane 3: Rabbit IgG instead of HA721240 in A375 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 26 seconds; ECL: K1801

Background References

1. Karamchandani JR. et al. Sox10 and S100 in the diagnosis of soft-tissue neoplasms. - Appl. Immunohistochem. Mol. Morphol. 2012;5:445-50
2. Nonaka D. et al. Sox10: a pan-schwannian and melanocytic marker. - Am. J. Surg. Pathol. 2008;9:1291-8
3. Ramos-Herberth FI. et al. SOX10 immunostaining distinguishes desmoplastic melanoma from excision scar. - J. Cutan. Pathol. 2010;9:944-52