Anti-Otx2 Antibody [PSH01-61]

HA721704

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell, FC, IHC-Fr, IF-Tissue
Molecular Wt:	Predicted band size: 32 kDa
Clone number:	PSH01-61

Description: Homeobox protein OTX1 is a protein that in humans is encoded by the OTX1 gene. This gene encodes a member of the bicoid sub-family of homeodomain-containing transcription factors. The encoded protein acts as a transcription factor and may play a role in brain and sensory organ development. The Otx gene is active in the region of the first gill arch, which is related to the upper and lower jaw and two of the bones of the ear. A similar protein in mice is required for proper brain and sensory organ development and can cause epilepsy.

Immunogen: Recombinant protein within human OTX2 aa 1-289.

Positive control: 293T transfected with FLAG-tagged Otx2 cell lysate, mouse eyeball tissue lysate, rat embryo tissue lysate, rat eyeball tissue lysate, mouse embryo tissue, mouse eye tissue, rat eye tissue, 293T overexpress with Otx2, E14.5 mouse embryo tissue.

Subcellular location: Nucleus.

Database links: SwissProt: P32243 Human | P80206 Mouse | Q64201 Rat

Recommended Dilutions:

WB	1:1,000-1:5,000
IHC-P	1:200-1:1,000
IF-Cell	1:10,000
FC	1:1,000
IHC-Fr	1:500
IF-Tissue	1:100

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

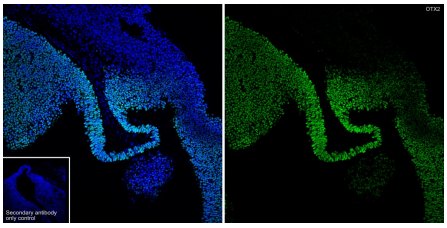

Fig1: Immunofluorescence analysis of frozen E14.5 mouse embryo tissue with Rabbit anti-Otx2 antibody (HA721704) at 1/200 dilution.

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 (HA721704, green) at 1/200 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).

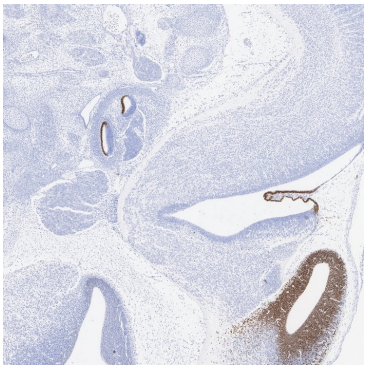

Fig2: Immunohistochemical analysis of paraffin-embedded mouse embryo tissue with Rabbit anti-Otx2 antibody (HA721704) at 1/1,000 dilution.

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 (HA721704) 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.

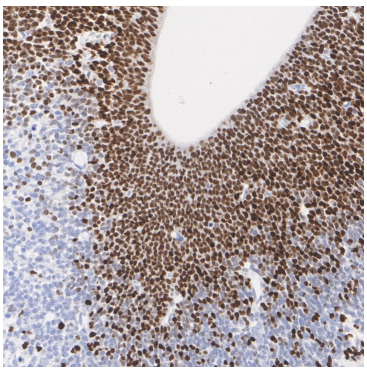

Fig3: Immunohistochemical analysis of paraffin-embedded mouse embryo tissue with Rabbit anti-Otx2 antibody (HA721704) at 1/1,000 dilution.

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 (HA721704) 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.

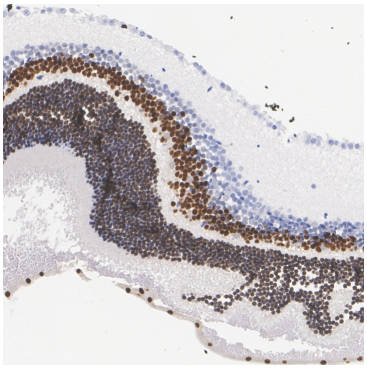

Fig4: Immunohistochemical analysis of paraffin-embedded mouse eye tissue with Rabbit anti-Otx2 antibody (HA721704) at 1/1,000 dilution.

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 (HA721704) 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.

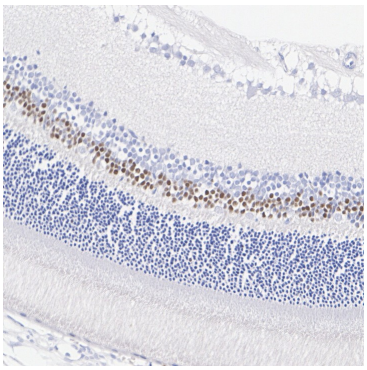

Fig5: Immunohistochemical analysis of paraffin-embedded rat eye tissue with Rabbit anti-Otx2 antibody (HA721704) at 1/200 dilution.

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 (HA721704) 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.

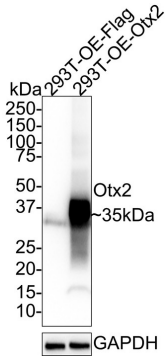

Fig6: Western blot analysis of Otx2 on different lysates with Rabbit anti-Otx2 antibody (HA721704) at 1/1,000 dilution.

Lane 1: 293T transfected with FLAG-tagged empty control cell lysate
Lane 2: 293T transfected with FLAG-tagged Otx2 cell lysate

Lysates/proteins at 30 µg/Lane.

Predicted band size: 32 kDa
Observed band size: 35 kDa

Exposure time: 24 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 (HA721704) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/100,000 dilution was used for 1 hour at room temperature.

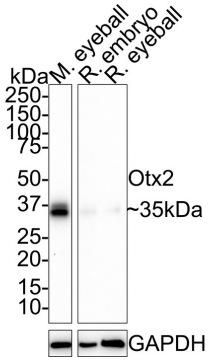

Fig7: Western blot analysis of Otx2 on different lysates with Rabbit anti-Otx2 antibody (HA721704) at 1/1,000 dilution.

Lane 1: Mouse eyeball tissue lysate (40 µg/Lane)
Lane 2: Rat embryo tissue lysate (40 µg/Lane)
Lane 3: Rat eyeball tissue lysate (40 µg/Lane)

Predicted band size: 32 kDa
Observed band size: 35 kDa

Exposure time: 5 minutes 10 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 (HA721704) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

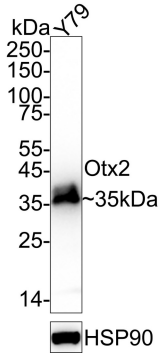

Fig8: Western blot analysis of Otx2 on Y79 cell lysates with Rabbit anti-Otx2 antibody (HA721704) at 1/5,000 dilution.

Lysates/proteins at 20 µg/Lane.

Predicted band size: 32 kDa
Observed band size: 35 kDa

Exposure time: 9 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 (HA721704) at 1/5,000 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.

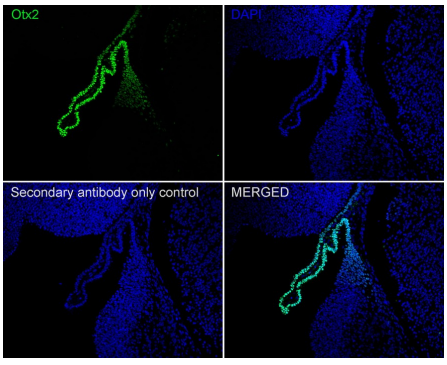

Fig9: Immunofluorescence analysis of paraffin-embedded mouse embryo tissue labeling Otx2 with Rabbit anti-Otx2 antibody (HA721704) at 1/100 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA721704, green) at 1/100 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).

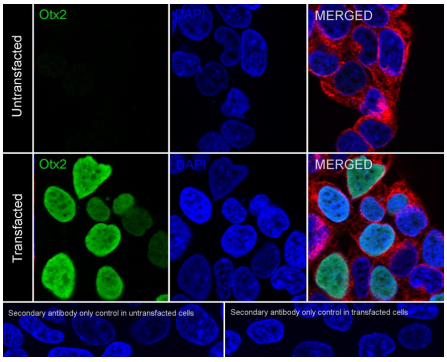

Fig10: Immunocytochemistry analysis of 293T overexpress with or without Otx2 cells labeling Otx2 with Rabbit anti-Otx2 antibody (HA721704) at 1/10,000 dilution.

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-Otx2 antibody (HA721704) at 1/10,000 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 (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.

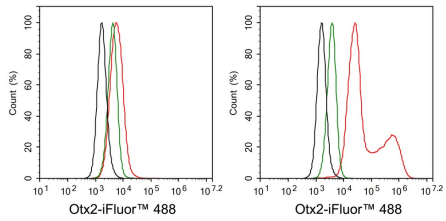

Fig11: Flow cytometric analysis of 293T overexpress with or without Otx2 cells labeling Otx2.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721704, 1μg/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).

Background References

1. Zhou L et al. OTX1 promotes tumorigenesis and progression of cervical cancer by regulating the Wnt signaling pathway. Oncol Rep. 2022 Nov
2. Ibad RT et al. OTX2 stimulates adult retinal ganglion cell regeneration. Neural Regen Res. 2022 Mar