Anti-Beta III Tubulin Antibody [PSH15-32] - BSA and Azide free

HA751554

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Tissue, IF-Cell, FC, IP
Molecular Wt:	Predicted band size: 50 kDa
Clone number:	PSH15-32

Description: Tubulin beta-3 chain, Class III β-tubulin, βIII-tubulin (β3-tubulin) or β-tubulin III, is a microtubule element of the tubulin family found almost exclusively in neurons, and in testis cells. In humans, it is encoded by the TUBB3 gene. Forebrain neuronal culture after 40 days of differentiation from induced human pluripotent stem cells. Class III β-tubulin is one of the seven β-tubulin isotypes identified in the human genome, predominantly in neurons and the testis. It is conditionally expressed in a number of other tissues after exposure to a toxic microenvironment featured by hypoxia and poor nutrient supply. Posttranslational changes including phosphorylation and glycosylation are required for functional activity. Class III β-tubulin's role in neural development has warranted its use as an early biomarker of neural cell differentiation from multi potent progenitors. TUBB3 inactivation impairs neural progenitor proliferation. Rescue experiments demonstrate the non-interchangeability of TUBB3 with other classes of β-tubulins which cannot restore the phenotype resulting from TUBB3 inactivation. Congenital neurologic syndromes associated with TUBB3 missense mutations demonstrate the critical importance of class III β-tubulin for normal neural development.

Immunogen: Synthetic peptide within mouse Beta III Tubulin aa 401-450.

Positive control: SH-SY5Y cell lysate, U-87 MG cell lysate, Neuro-2a cell lysate, Mouse brain tissue lysate, Rat brain tissue lysate, mouse neuron cells, human cerebellum tissue, mouse E14.5 embryo tissue, mouse cerebellum tissue, rat E14.5 embryo tissue, rat cerebellum tissue, SH-SY5Y, Neuro-2a.

Subcellular location: Cytoplasm, cytoskeleton, growth cone, lamellipodium, filopodium.

Database links: SwissProt: Q13509 Human | Q9ERD7 Mouse | Q4QRB4 Rat

Recommended Dilutions:

WB	1:2,500
IHC-P	1:2,000-1:4,000
IF-Tissue	1:400
IF-Cell	1:500
FC	1:1,000-1:10,000
IP	1-2μg/sample

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

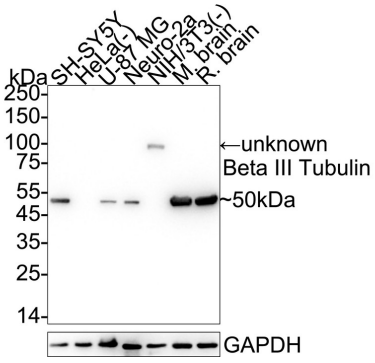

Fig1: Western blot analysis of Beta III Tubulin on different lysates with Rabbit anti-Beta III Tubulin antibody (HA751554) at 1/2,500 dilution.

Lane 1: SH-SY5Y cell lysate (15 µg/Lane)
Lane 2: HeLa cell lysate (negative) (15 µg/Lane)
Lane 3: U-87 MG cell lysate (15 µg/Lane)
Lane 4: Neuro-2a cell lysate (15 µg/Lane)
Lane 5: NIH/3T3 cell lysate (negative) (15 µg/Lane)
Lane 6: Mouse brain tissue lysate (20 µg/Lane)
Lane 7: Rat brain tissue lysate (20 µg/Lane)

Predicted band size: 50 kDa
Observed band size: 50 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 (HA751554) at 1/2,500 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.

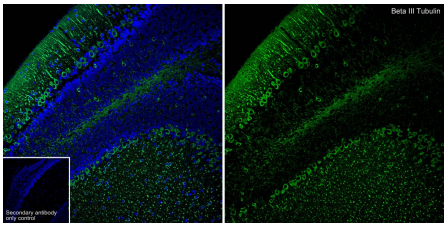

Fig2: Application: IF-Tissue

Species: Mouse

Site: cerebellum

Sample: Paraffin-embedded section

Antibody concentration: 1/400

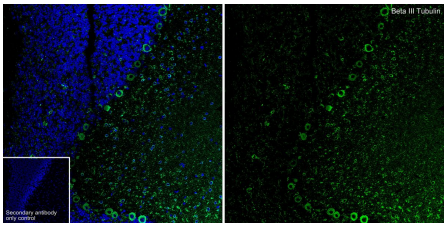

Fig3: Application: IF-Tissue

Species: Rat

Site: cerebellum

Sample: Paraffin-embedded section

Antibody concentration: 1/400

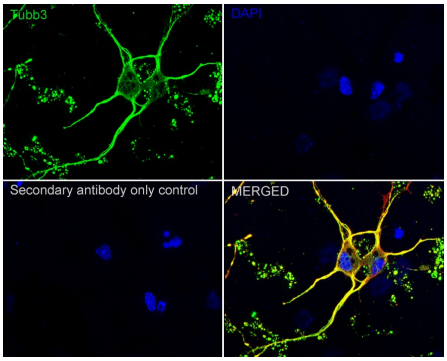

Fig4: Immunocytochemistry analysis of mouse neuron cells labeling Beta III Tubulin with Rabbit anti-Beta III Tubulin antibody (HA751554) at 1/500 dilution.

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-Beta III Tubulin antibody (HA751554) 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 (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.

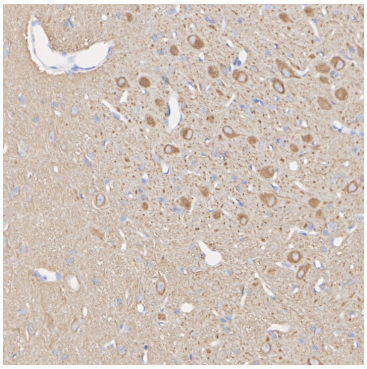

Fig5: Immunohistochemical analysis of paraffin-embedded human cerebellum tissue with Rabbit anti-Beta III Tubulin antibody (HA751554) at 1/4,000 dilution.

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 (HA751554) 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.

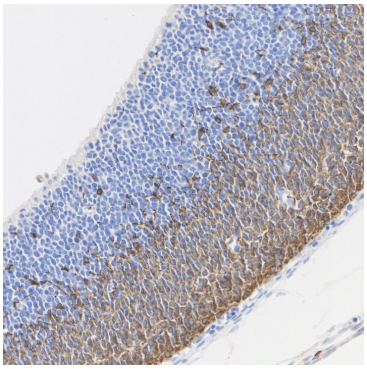

Fig6: Immunohistochemical analysis of paraffin-embedded mouse E14.5 embryo tissue with Rabbit anti-Beta III Tubulin antibody (HA751554) at 1/4,000 dilution.

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 (HA751554) 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.

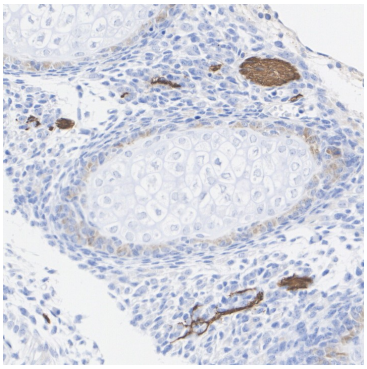

Fig7: Immunohistochemical analysis of paraffin-embedded mouse E14.5 embryo tissue with Rabbit anti-Beta III Tubulin antibody (HA751554) at 1/4,000 dilution.

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 (HA751554) 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.

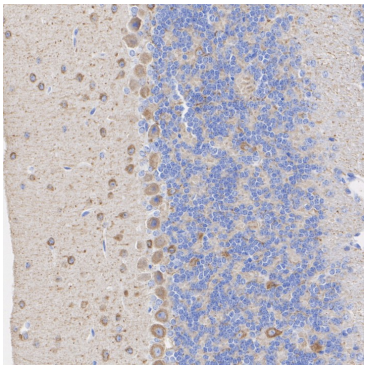

Fig8: Immunohistochemical analysis of paraffin-embedded mouse cerebellum tissue with Rabbit anti-Beta III Tubulin antibody (HA751554) at 1/4,000 dilution.

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 (HA751554) 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.

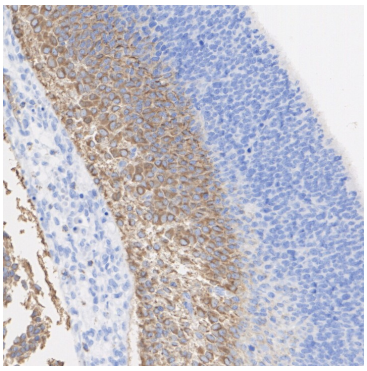

Fig9: Immunohistochemical analysis of paraffin-embedded rat E14.5 embryo tissue with Rabbit anti-Beta III Tubulin antibody (HA751554) at 1/4,000 dilution.

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 (HA751554) 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.

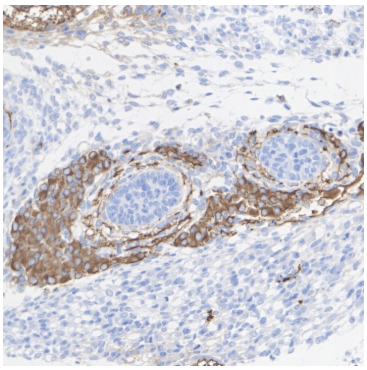

Fig10: Immunohistochemical analysis of paraffin-embedded rat E14.5 embryo tissue with Rabbit anti-Beta III Tubulin antibody (HA751554) at 1/4,000 dilution.

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 (HA751554) 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.

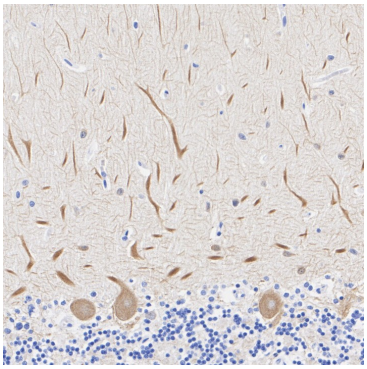

Fig11: Immunohistochemical analysis of paraffin-embedded rat cerebellum tissue with Rabbit anti-Beta III Tubulin antibody (HA751554) at 1/4,000 dilution.

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 (HA751554) 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.

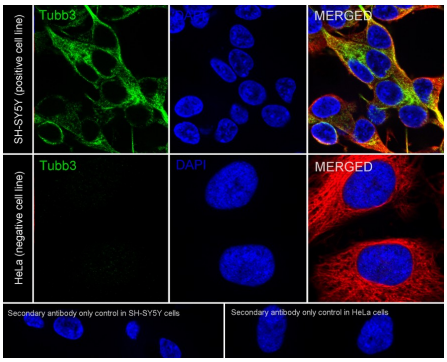

Fig12: Immunocytochemistry analysis of SH-SY5Y (positive) and HeLa (negative) labeling Beta III Tubulin with Rabbit anti-Beta III Tubulin antibody (HA751554) at 1/500 dilution.

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-Beta III Tubulin antibody (HA751554) 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 (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.

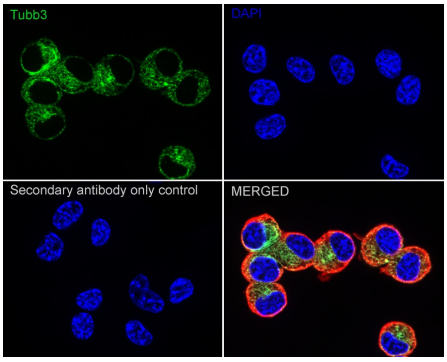

Fig13: Immunocytochemistry analysis of Neuro-2a cells labeling Beta III Tubulin with Rabbit anti-Beta III Tubulin antibody (HA751554) at 1/500 dilution.

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-Beta III Tubulin antibody (HA751554) 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 (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.

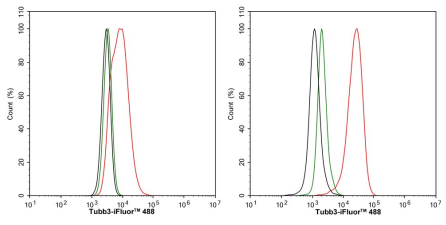

Fig14: Flow cytometric analysis of HeLa (left, negative) and SH-SY5Y (right, positive) cells labeling Beta III Tubulin.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA751554, 1/10,000) (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).

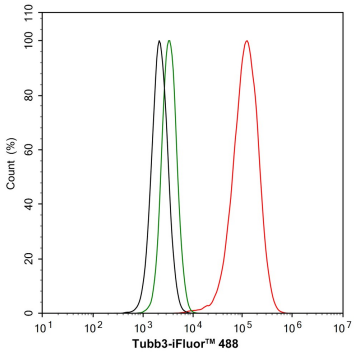

Fig15: Flow cytometric analysis of Neuro-2a cells labeling Beta III Tubulin.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA751554, 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).

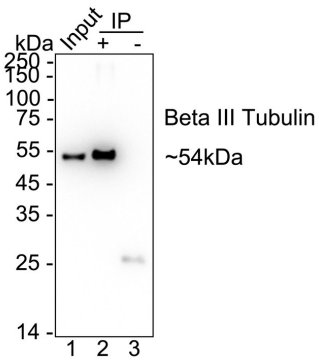

Fig16: Beta III Tubulin was immunoprecipitated from 0.2 mg mouse brain tissue lysate with HA751554 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA751554 at 1/500 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: mouse brain tissue lysate (input)
Lane 2: HA751554 IP in mouse brain tissue lysate
Lane 3: Rabbit IgG instead of HA751554 in mouse brain tissue lysate

Blocking/Dilution buffer: primary antibody dilution (K1803)
Exposure time: 7 seconds; ECL: K1801

Background References

1. Puri D et al. TUBB3 and KIF21A in neurodevelopment and disease. Front Neurosci. 2023 Aug
2. Jin S et al. TUBB3 M323V Syndrome Presents with Infantile Nystagmus. Genes (Basel). 2021 Apr