Anti-alpha Tubulin Antibody [PSH02-95]

HA721914

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell, FC, IP
Molecular Wt:	Predicted band size: 50 kDa
Clone number:	PSH02-95

Description: The cytoskeleton consists of three types of cytosolic fibers: microtubules, microfilaments (actin filaments), and intermediate filaments. Globular tubulin subunits comprise the microtubule building block, with α/β-tubulin heterodimers forming the tubulin subunit common to all eukaryotic cells. Acetylation of alpha chains at Lys-40 stabilizes microtubules and affects affinity and processivity of microtubule motors. This modification has a role in multiple cellular functions, ranging from cell motility, cell cycle progression or cell differentiation to intracellular trafficking and signaling.

Immunogen: Synthetic peptide within Human alpha Tubulin aa 402-451 / 451.

Positive control: HeLa cell lysate, Daudi cell lysate, Jurkat cell lysate, A431 cell lysate, K-562 cell lysate, 293T cell lysate, NIH/3T3 cell lysate, PC-12 cell lysate, Rat brain tissue lysate, Mouse brain tissue lysate, Mouse spleen tissue lysate, human tonsil tissue, rat brain tissue, Daudi, K-562, HeLa.

Subcellular location: Cytoplasm, Cytoskeleton, Microtubule.

Database links: SwissProt: P68363 Human | P68368 Mouse | Q5XIF6 Rat

Recommended Dilutions:

WB	1:1,000-5,000
IHC-P	1:1,000
IF-Cell	1:100
FC	1:1,000
IP	1-2μg/sample

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

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

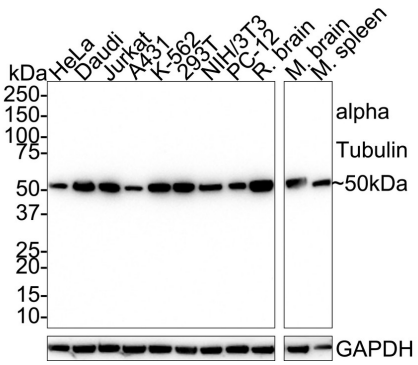

Fig1: Western blot analysis of alpha Tubulin on different lysates with Rabbit anti-alpha Tubulin antibody (HA721914) at 1/5,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)
Lane 2: Daudi cell lysate (20 µg/Lane)
Lane 3: Jurkat cell lysate (20 µg/Lane)
Lane 4: A431 cell lysate (20 µg/Lane)
Lane 5: K-562 cell lysate (20 µg/Lane)
Lane 6: 293T cell lysate (20 µg/Lane)
Lane 7: NIH/3T3 cell lysate (20 µg/Lane)
Lane 8: PC-12 cell lysate (20 µg/Lane)
Lane 9: Rat brain tissue lysate (40 µg/Lane)
Lane 10: Mouse brain tissue lysate (40 µg/Lane)
Lane 11: Mouse spleen tissue lysate (40 µg/Lane)

Predicted band size: 50 kDa
Observed band size: 50 kDa

Exposure time: 3 minutes; 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 (HA721914) 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.

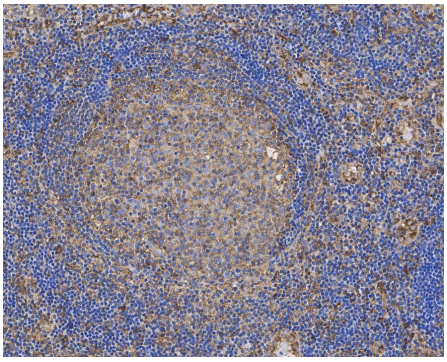

Fig2: Immunofluorescence analysis of paraffin-embedded human tonsil tissue labeling alpha Tubulin with Rabbit anti-alpha Tubulin antibody (HA721914) at 1/1,000 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA721914, green) at 1/1,000 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).

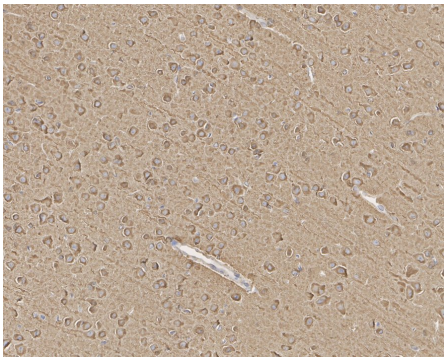

Fig3: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-alpha Tubulin antibody (HA721914) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) 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 (HA721914) 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.

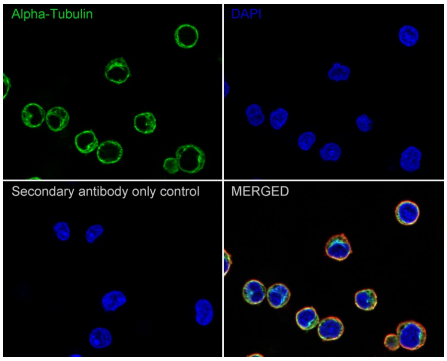

Fig4: Immunocytochemistry analysis of Daudi cells labeling alpha Tubulin with Rabbit anti-alpha Tubulin antibody (HA721914) at 1/100 dilution.

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-alpha Tubulin antibody (HA721914) 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.

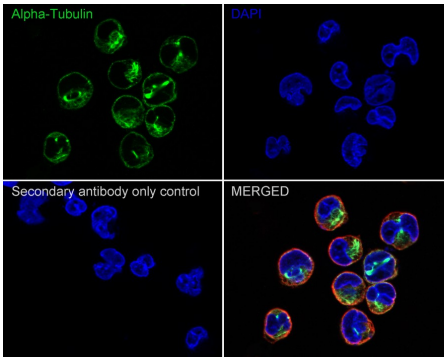

Fig5: Immunocytochemistry analysis of K-562 cells labeling alpha Tubulin with Rabbit anti-alpha Tubulin antibody (HA721914) at 1/100 dilution.

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-alpha Tubulin antibody (HA721914) 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.

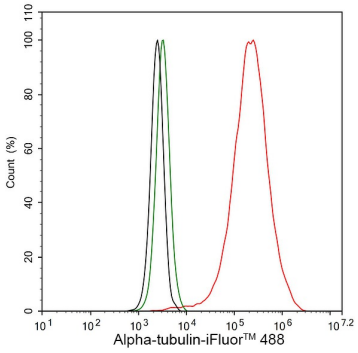

Fig6: Flow cytometric analysis of HeLa cells labeling alpha Tubulin.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721914, 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).

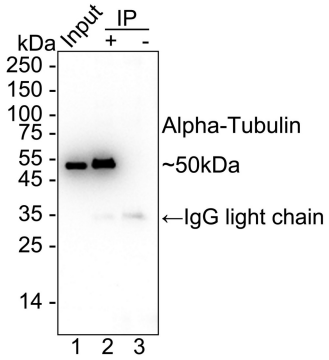

Fig7: alpha Tubulin was immunoprecipitated from 0.2 mg HeLa cell lysate with HA721914 at 2 μg/10 μl beads. Western blot was performed from the immunoprecipitate using HA721914 at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: HA721914 IP in HeLa cell lysate
Lane 3: Rabbit IgG instead of HA721914 in HeLa cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 2 seconds; ECL: K1801

Background References

1. Girão H et al. alpha-tubulin detyrosination fine-tunes kinetochore-microtubule attachments. Nat Commun. 2024 Nov
2. Wethekam LC et al. alpha-tubulin regulation by 5' introns in Saccharomyces cerevisiae. Genetics. 2023 Dec