Anti-alpha Tubulin 4A Antibody [JM73-24]

ET1705-31

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC
Molecular Wt:	Predicted band size: 50 kDa
Clone number:	JM73-24

Description: Tubulin alpha-4A chain is a protein that in humans is encoded by the TUBA4A gene. Microtubules of the eukaryotic cytoskeleton perform essential and diverse functions and are composed of a heterodimer of alpha and beta tubulin. The genes encoding these microtubule constituents are part of the tubulin superfamily, which is composed of six distinct families. Genes from the alpha, beta and gamma tubulin families are found in all eukaryotes. The alpha and beta tubulins represent the major components of microtubules, while gamma tubulin plays a critical role in the nucleation of microtubule assembly. There are multiple alpha and beta tubulin genes and they are highly conserved among and between species. This gene encodes an alpha tubulin that is a highly conserved homolog of a rat testis-specific alpha tubulin.

Immunogen: Synthetic peptide within Human alpha Tubulin 4A aa 399-448 / 448.

Positive control: HeLa cell lysate, PC-12 cell lysate, Mouse brain tissue lysate, Rat brain tissue lysate, HeLa, human tonsil tissue, human thyroid tissue, mouse brain tissue, human placenta tissue, mouse testis tissue.

Subcellular location: Cytoskeleton.

Database links: SwissProt: P68366 Human | P68368 Mouse | Q5XIF6 Rat

Recommended Dilutions:

WB	1:500-1:2,000
IF-Cell	1:500
IF-Tissue	1:100-1:200
IHC-P	1:100-1:200
FC	1:1,000

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

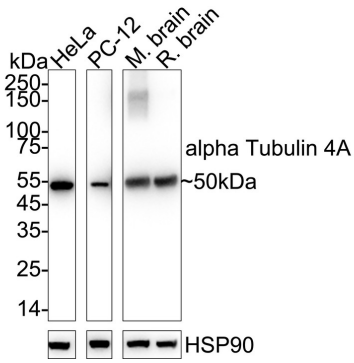

Fig1: Western blot analysis of alpha Tubulin 4A on different lysates with Rabbit anti-alpha Tubulin 4A antibody (ET1705-31) at 1/1,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)
Lane 2: PC-12 cell lysate (20 µg/Lane)
Lane 3: Mouse brain tissue lysate (40 µg/Lane)
Lane 4: Rat brain tissue lysate (40 µg/Lane)

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

Exposure time: Lane 1-2: 8 seconds; Lane 3-4: 2 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 (ET1705-31) 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.

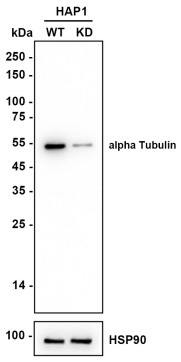

Fig2: Western blot analysis of alpha Tubulin 4A on different lysates with Rabbit anti-alpha Tubulin 4A antibody (ET1705-31) at 1/2,000 dilution.

Lane 1: HAP1-parental cell lysate
Lane 2: HAP1-alpha Tubulin 4A KD cell lysate

Lysates/proteins at 10 µg/Lane.

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

Exposure time: 10 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 (ET1705-31) at 1/2,000 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.

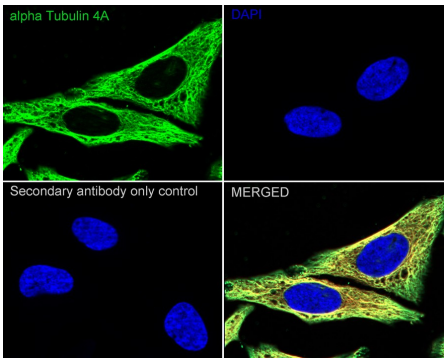

Fig3: Immunocytochemistry analysis of HeLa cells labeling alpha Tubulin 4A with Rabbit anti-alpha Tubulin 4A antibody (ET1705-31) at 1/500 dilution.

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-alpha Tubulin 4A antibody (ET1705-31) 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.

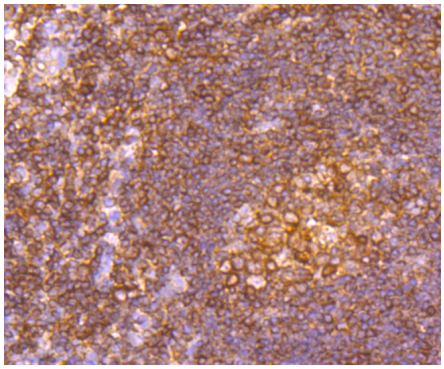

Fig4: Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-alpha Tubulin 4A antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1705-31, 1/50) for 30 minutes 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.

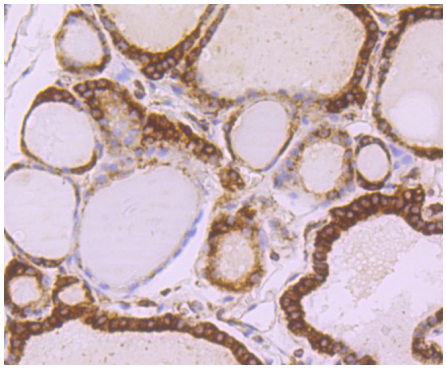

Fig5: Immunohistochemical analysis of paraffin-embedded human thyroid tissue using anti-alpha Tubulin 4A antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1705-31, 1/50) for 30 minutes 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.

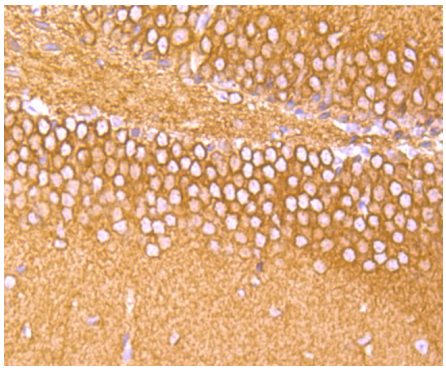

Fig6: Immunohistochemical analysis of paraffin-embedded mouse brain tissue using anti-alpha Tubulin 4A antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1705-31, 1/50) for 30 minutes 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.

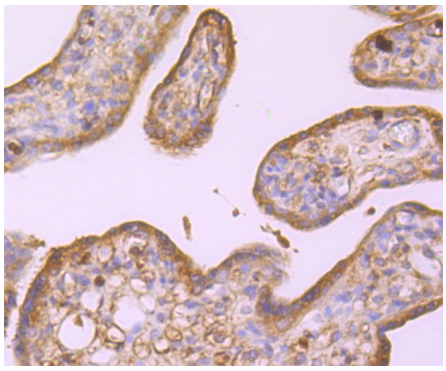

Fig7: Immunohistochemical analysis of paraffin-embedded human placenta tissue using anti-alpha Tubulin 4A antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1705-31, 1/50) for 30 minutes 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.

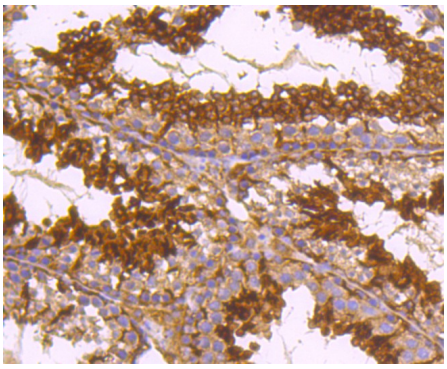

Fig8: Immunohistochemical analysis of paraffin-embedded mouse testis tissue using anti-alpha Tubulin 4A antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1705-31, 1/50) for 30 minutes 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.

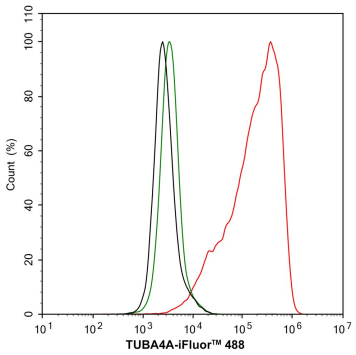

Fig9: Flow cytometric analysis of HeLa cells labeling alpha Tubulin 4A.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1705-31, 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).

Background References

1. Monterisi S et al. PDE2A2 regulates mitochondria morphology and apoptotic cell death via local modulation of cAMP/PKA signalling. Elife 6. pii: e21374 (2017).
2. Jiang YF et al. Electron tomographic analysis reveals ultrastructural features of mitochondrial cristae architecture which reflect energetic state and aging. Sci Rep 7:45474 (2017).