Anti-alpha Tubulin Antibody

ER130905

Product Type:	Rabbit polyclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Zebrafish
Applications:	WB, IHC-P, FC, IF-Cell, IF-Tissue
Molecular Wt:	Predicted band size: 50 kDa

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: NIH/3T3 cell lysate, HepG2 cell lysate, PC-12 cell lysate, Hela cell lysate, HepG2, Hela, Neuro-2a, C6, human stomach tissue, human tonsil tissue, mouse pancreas tissue, rat large intestine tissue.

Subcellular location: Cytoplasm, Cytoskeleton, Microtubule.

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

Recommended Dilutions:

WB	1:1,000-1:10,000
IHC-P	1:200-1:500
FC	1:50-1:100
IF-Cell	1:200-1:500
IF-Tissue	1:200

Storage Buffer: 1*PBS (pH7.4), 0.2% 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: Immunogen affinity purified.

Images

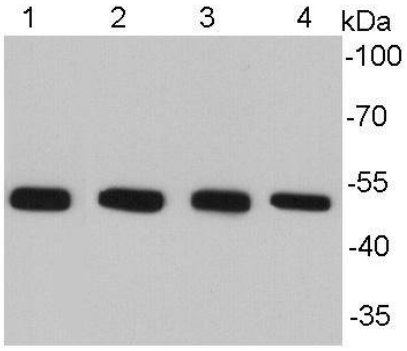

Fig1: Western blot analysis of alpha Tubulin on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ER130905, 1/10,000) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.
Positive control:
Lane 1: NIH/3T3 cell lysate
Lane 2: HepG2 cell lysate
Lane 3: PC-12 cell lysate
Lane 4: Hela cell lysate

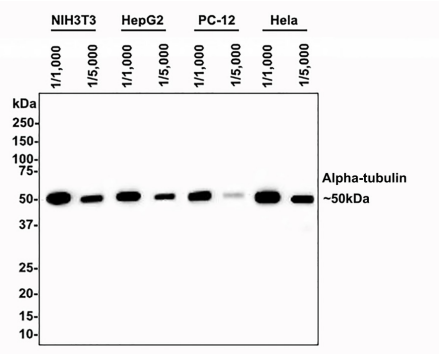

Fig2: Western blot analysis of alpha Tubulin on different lysates with Rabbit anti-alpha Tubulin antibody (ER130905) at different dilutions.

Lane 1/2: NIH3T3 cell lysate at 1/1,000 and 1/5,000 dilution
Lane 3/4: HepG2 cell lysate at 1/1,000 and 1/5,000 dilution
Lane 5/6: PC-12 cell lysate at 1/1,000 and 1/5,000 dilution
Lane 7/8: Hela cell lysate at 1/1,000 and 1/5,000 dilution

Lysates/proteins at 10 µg/Lane.

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

Exposure time: 1 minute;

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 (ER130905) at different dilutions were 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.

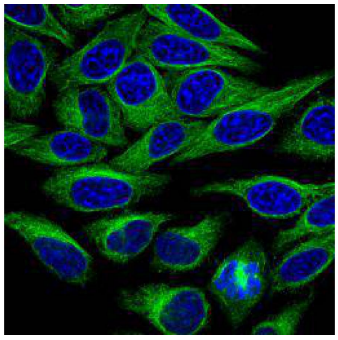

Fig3: ICC staining of alpha Tubulin in HepG2 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (ER130905, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

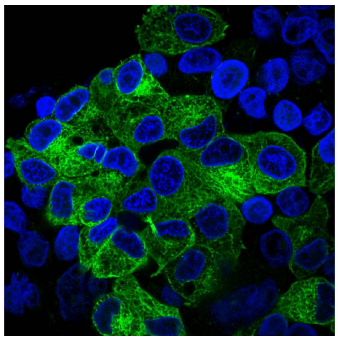

Fig4: ICC staining of alpha Tubulin in Hela cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (ER130905, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

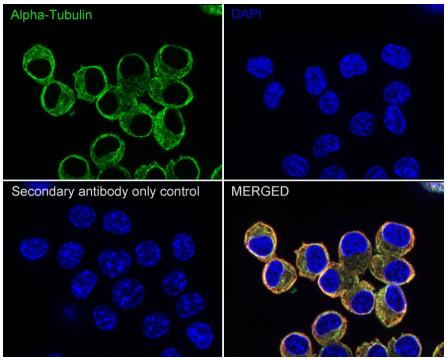

Fig5: Immunocytochemistry analysis of Neuro-2a cells labeling alpha Tubulin with Rabbit anti-alpha Tubulin antibody (ER130905) at 1/250 dilution.

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 (ER130905) at 1/250 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.

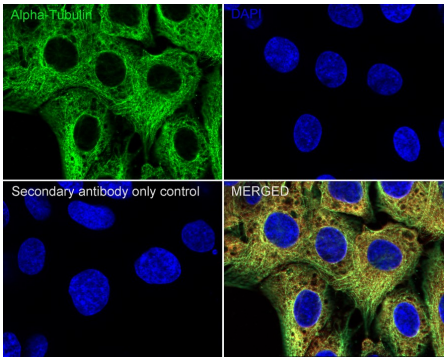

Fig6: Immunocytochemistry analysis of C6 cells labeling alpha Tubulin with Rabbit anti-alpha Tubulin antibody (ER130905) at 1/250 dilution.

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 (ER130905) at 1/250 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.

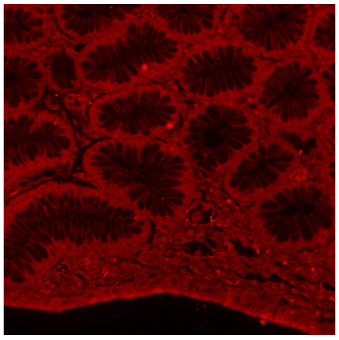

Fig7: Immunofluorescence staining of paraffin-embedded human stomach tissue using anti-alpha Tubulin antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with ER130905 at 1/50 dilution for 10 hours at 4℃ and detected using Alexa Fluor® 594 conjugate-Goat anti-Rabbit IgG (H+L) Secondary Antibody at a dilution of 1:500 for 1 hour at room temperature.

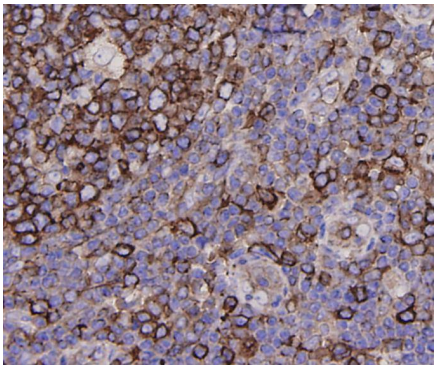

Fig8: Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-alpha Tubulin antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ER130905, 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.

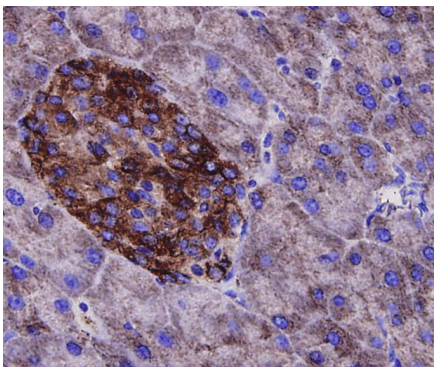

Fig9: Immunohistochemical analysis of paraffin-embedded mouse pancreas tissue using anti-alpha Tubulin antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ER130905, 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.

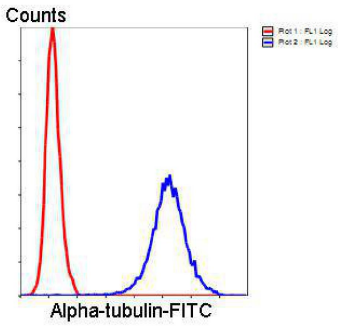

Fig10: Flow cytometric analysis of alpha Tubulin was done on Hela cells. The cells were fixed, permeabilized and stained with the primary antibody (ER130905, 1/50) (blue). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; red).

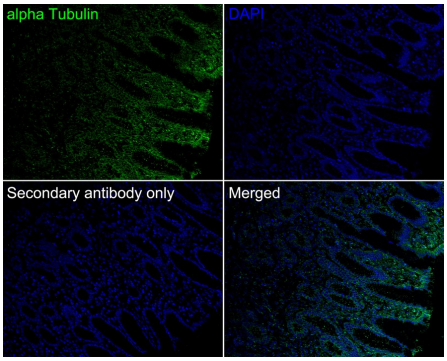

Fig11: Immunofluorescence analysis of paraffin-embedded human stomach tissue labeling alpha Tubulin with Rabbit anti-alpha Tubulin antibody (ER130905) at 1/200 dilution.

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 (ER130905, 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).

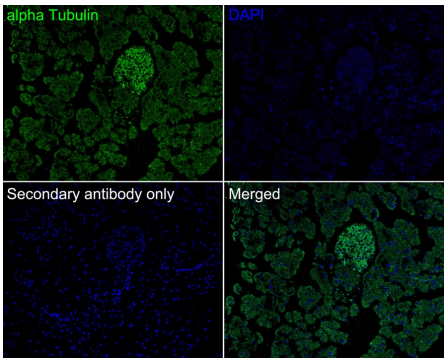

Fig12: Immunofluorescence analysis of paraffin-embedded mouse pancreas tissue labeling alpha Tubulin with Rabbit anti-alpha Tubulin antibody (ER130905) at 1/200 dilution.

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 (ER130905, 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).

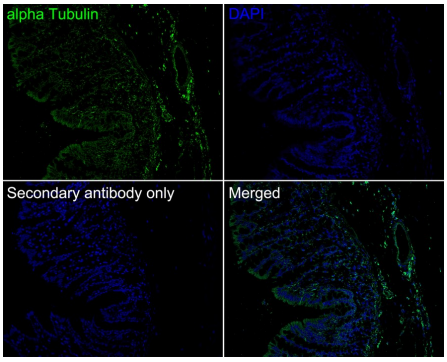

Fig13: Immunofluorescence analysis of paraffin-embedded rat large intestine tissue labeling alpha Tubulin with Rabbit anti-alpha Tubulin antibody (ER130905) at 1/200 dilution.

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 (ER130905, 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).

Background References

1. "C-terminal fragments of alpha- and beta-tubulin form amyloid fibrils in vitro and associate with amyloid deposits of familial cerebral amyloid angiopathy, British type." Baumann M.H., Wisniewski T., Levy E., Plant G.T., Ghiso J. Biochem. Biophys. Res. Commun. 219:238-242(1996)
2. "Tubulin polyglutamylase enzymes are members of the TTL domain protein family." Janke C., Rogowski K., Wloga D., Regnard C., Kajava A.V., Strub J.-M., Temurak N., van Dijk J., Boucher D., van Dorsselaer A., Suryavanshi S., Gaertig J., Edde B. Science 308:1758-1762(2005)
3. "Evolutionary divergence of enzymatic mechanisms for posttranslational polyglycylation." Rogowski K., Juge F., van Dijk J., Wloga D., Strub J.-M., Levilliers N., Thomas D., Bre M.-H., Van Dorsselaer A., Gaertig J., Janke C. Cell 137:1076-1087(2009)