abcam

Product datasheet

Anti-alpha Tubulin antibody - Loading Control ab89984

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Overview

Product name	Anti-alpha Tubulin antibody - Loading Control
Description	Chicken polyclonal to alpha Tubulin - Loading Control
Host species	Chicken
Tested applications	**Suitable for:** WB, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Chicken, Cow, Monkey
Immunogen	Synthetic peptide corresponding to Human alpha Tubulin aa 1-100 conjugated to keyhole limpet haemocyanin. (Peptide available as ab23537)
Positive control	This antibody gave a positive signal in the following whole cell lysates: HeLa; HEK293; HepG2; Caco2; HCT116; NIH 3T3; PC12; ICC/IF: Caco-2 cells, NIH3T3 cells, SV40LT-SMC cells

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituents: 1% BSA, PBS This product may contain up to 3% BSA depending on the batch. For specific batch formulations please contact us.
Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgY

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab89984 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★☆ (1)	Use a concentration of 1 µg/ml. Detects a band of approximately 53 kDa (predicted molecular weight: 50 kDa).
ICC/IF	★★★★☆ (4)	Use a concentration of 1 - 5 µg/ml.

Target

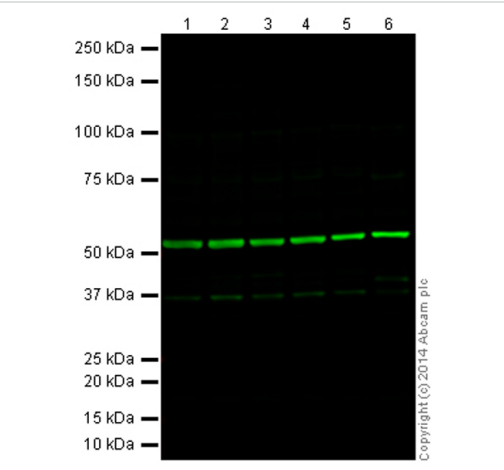
Function Tubulin is the major constituent of microtubules. It binds two moles of GTP, one at an exchangeable site on the beta chain and one at a non-exchangeable site on the alpha chain.

Sequence similarities Belongs to the tubulin family.

Post-translational modifications Some glutamate residues at the C-terminus are polyglutamylated. This modification occurs exclusively on glutamate residues and results in polyglutamate chains on the gamma-carboxyl group. Also monoglycylated but not polyglycylated due to the absence of functional TTLL10 in human. Monoglycylation is mainly limited to tubulin incorporated into axonemes (cilia and flagella) whereas glutamylation is prevalent in neuronal cells, centrioles, axonemes, and the mitotic spindle. Both modifications can coexist on the same protein on adjacent residues, and lowering glycylation levels increases polyglutamylation, and reciprocally. The precise function of such modifications is still unclear but they regulate the assembly and dynamics of axonemal microtubules.
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.

Cellular localization Cytoplasm > cytoskeleton.

Images



Western blot - Anti-alpha Tubulin antibody - Loading Control (ab89984)

All lanes : Anti-alpha Tubulin antibody - Loading Control (ab89984) at 1 µg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate

Lane 2 : HEK293 (Human embryonic kidney cell line) Whole Cell Lysate

Lane 3 : HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate

Lane 4 : Caco 2 (Human colonic carcinoma cell line) Whole Cell Lysate

Lane 5 : HCT 116 (Human Colorectal Carcinoma) Whole Cell Lysate

Lane 6 : NIH 3T3 (Mouse embryonic fibroblast cell line) Whole Cell

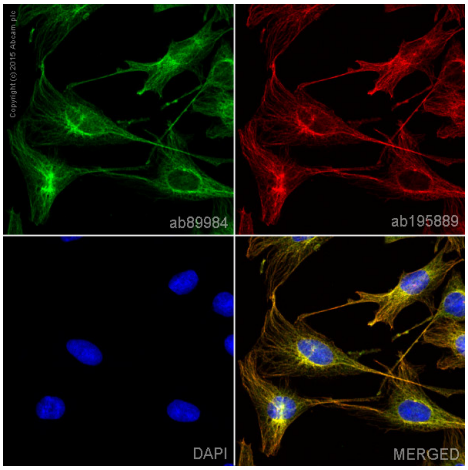
Lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Chicken IgY H&L (Alexa Fluor® 790) (ab175787) at 1/10000 dilution

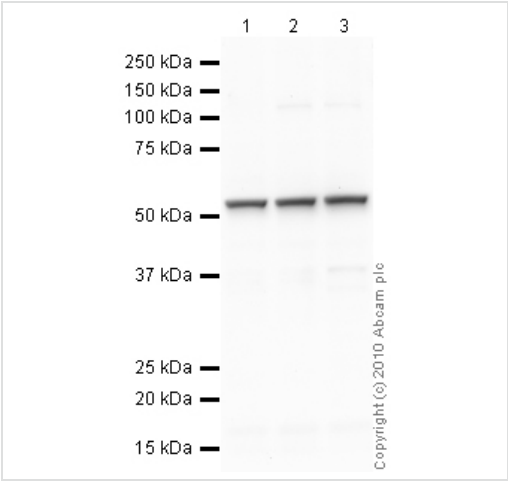
Predicted band size: 50 kDa
Observed band size: 52 kDa

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using Licor blocking buffer before being incubated with ab89984 overnight at 4°C. Antibody binding was detected using ab175787 at a 1:10,000 dilution for 1hr at room temperature and then imaged using the Licor Odyssey CLx.



Immunocytochemistry/ Immunofluorescence - Anti-alpha Tubulin antibody - Loading Control (ab89984)

ab89984 staining alpha Tubulin in SV40LT-SMC cells. The cells were fixed with 4% formaldehyde (10 min), permeabilized in 0.1% Triton X-100 for 5 minutes and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab89984 at a working concentration of 1µg/ml and ab195889, Mouse monoclonal [DM1A] to alpha Tubulin (Alexa Fluor® 594, shown in red) at 1/250 overnight at +4°C, followed by a further incubation at room temperature for 1h with an anti-chicken AlexaFluor® 488 (ab150173) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI. This product also gave a positive signal in 100% methanol (5 min) fixed SV40 cells under the same testing conditions.
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Western blot - Anti-alpha Tubulin antibody - Loading Control (ab89984)

All lanes : Anti-alpha Tubulin antibody - Loading Control (ab89984) at 1 µg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2 : NIH 3T3 (Mouse embryonic fibroblast cell line) Whole Cell Lysate
Lane 3 : PC12 (Rat adrenal pheochromocytoma cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

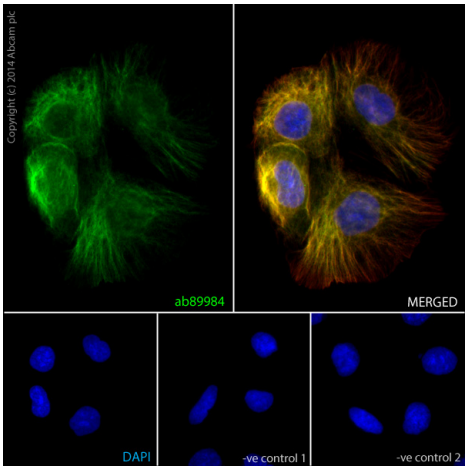
Secondary
All lanes : Goat polyclonal to Chicken IgY - H&L (HRP) at 1/3000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 50 kDa
Observed band size: 53 kDa
Additional bands at: 125 kDa, 37 kDa. We are unsure as to the identity of these extra bands.

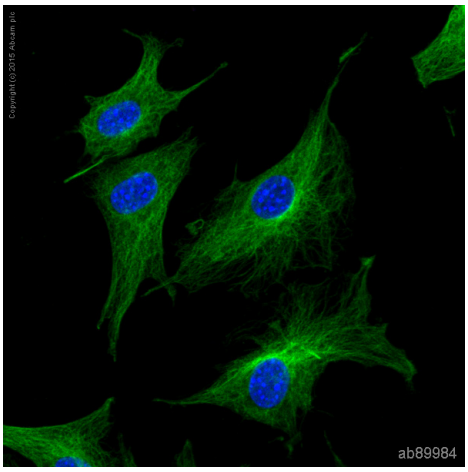
Exposure time: 30 seconds



Immunocytochemistry/ Immunofluorescence - Anti-alpha Tubulin antibody - Loading Control (ab89984)

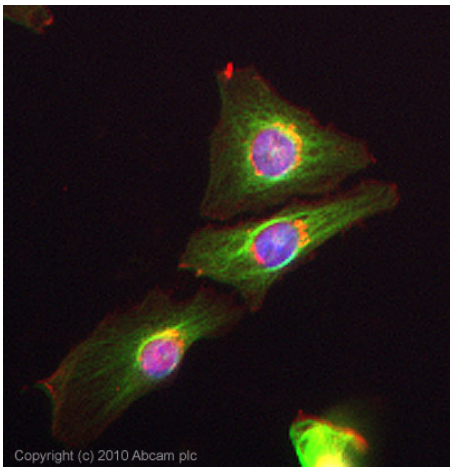
ab89984 staining alpha Tubulin in Caco-2 cells. The cells were fixed with 100% methanol (5min) and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with ab89984 at 5µg/ml and ab7291 at 1µg/ml overnight at +4°C, followed by a further incubation at room temperature for 1h with an AlexaFluor®488 Goat anti-Chicken secondary (ab150173) at 2 µg/ml (shown in green) and AlexaFluor®594 Goat anti-Mouse secondary (ab150120) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labelled in blue with DAPI.

Negative controls: 1– Rabbit primary antibody and anti-mouse secondary antibody; 2 – Mouse primary antibody and anti-rabbit secondary antibody. Controls 1 and 2 indicate that there is no unspecific reaction between primary and secondary antibodies used.



Immunocytochemistry/ Immunofluorescence - Anti-alpha Tubulin antibody - Loading Control (ab89984)

ab89984 staining Tubulin in NIH3T3 cells. The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab89984 at 5µg/ml (shown in green). Nuclear DNA was labelled with DAPI (shown in blue). Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunocytochemistry/ Immunofluorescence - Anti-alpha Tubulin antibody - Loading Control (ab89984)

ICC/IF image of ab89984 stained HeLa cells. The cells were 4% PFA fixed (10 min) and then incubated in 1%BSA / 10% normal Goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab89984 at 5ug overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 Goat anti- chicken IgY (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM. This antibody also gave a positive result in HepG2 PFA fixed cell types at 5ug/ml.

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