abcam

Product datasheet

Anti-alpha Tubulin (acetyl K40) antibody [6-11B-1] ab24610

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Overview

Product name	Anti-alpha Tubulin (acetyl K40) antibody [6-11B-1]
Description	Mouse monoclonal [6-11B-1] to alpha Tubulin (acetyl K40)
Host species	Mouse
Specificity	ab24610 detects acetylated alpha tubulin.
Tested applications	**Suitable for:** Flow Cyt, WB, IHC-P, ICC/IF, IHC-Fr
Species reactivity	**Reacts with:** Mouse, Rat, Sheep, Human, Monkey, Sea urchin **Predicted to work with:** Cow
Immunogen	Tissue, cells or virus corresponding to alpha Tubulin.
Epitope	The antibody recognizes an epitope located on the a3 isoform of Chlamydomonas axonemal a-tubulin, within four residues of Lys40 when this amino acid is acetylated.
Positive control	In Western Blot, this antibody gave a positive signal in mouse brain tissue lysate and in the following whole cell lysates: HeLa; NIH3T3; PC12.
General notes	Production of this antibody has been changed on 8th April 2016. This antibody is now purified from tissue culture supernatant. This shouldn't affect the use of this antibody but if you have any issues, please contact our Scientific Support team. This antibody binds to primary cilia, centrioles, mitotic spindles, midbodies and to subsets of cytoplasmic microtubules in 3T3 and HeLa cells.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.40 Preservative: 0.097% Sodium azide Constituent: PBS
Purity	Proprietary Purification
Purification notes	Purified from Tissue culture supernatant.
Clonality	Monoclonal

Clone number	6-11B-1
Isotype	IgG2b
Light chain type	kappa

Applications

Our Abpromise guarantee covers the use of **ab24610** in the following tested applications.

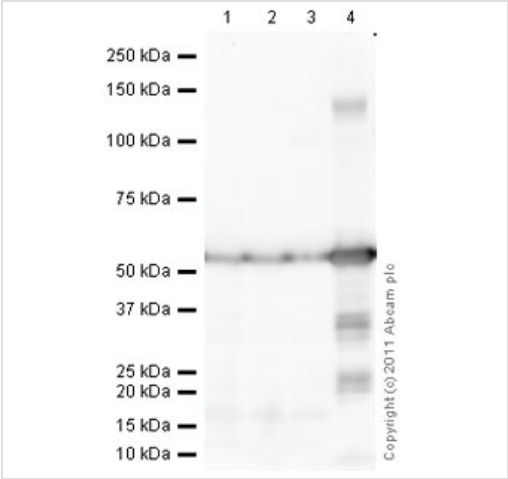
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt		Use 1µl for 10^6 cells. ab170192 - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody.
WB	★★★★☆	Use a concentration of 0.03 - 0.06 µg/ml. Detects a band of approximately 55 kDa (predicted molecular weight: 55 kDa).
IHC-P	★★★★★	Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
ICC/IF	★★★★★	Use at an assay dependent concentration.
IHC-Fr	★★★★☆	Use at an assay dependent concentration.

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 (acetyl K40) antibody [6-11B-1] (ab24610)

All lanes : Anti-alpha Tubulin (acetyl K40) antibody [6-11B-1] (ab24610) at 5 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
Lane 4 : Brain (Mouse) Tissue Lysate

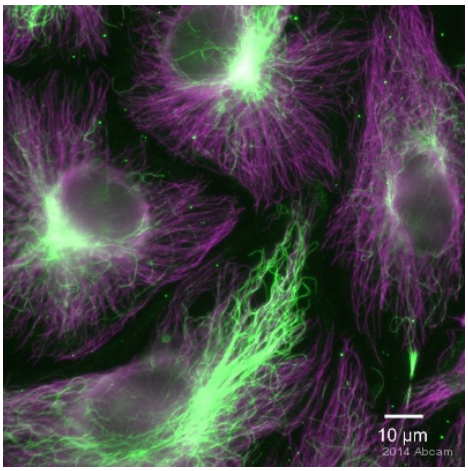
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Mouse IgG H&L (HRP) preadsorbed (ab97040) at 1/5000 dilution

Performed under reducing conditions.

Predicted band size: 55 kDa
Observed band size: 55 kDa
Additional bands at: 140 kDa, 25 kDa, 35 kDa. We are unsure as to the identity of these extra bands.

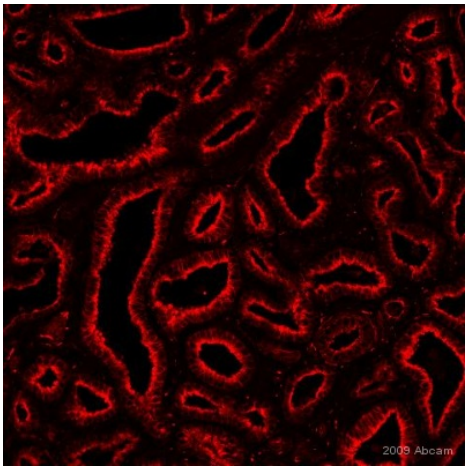
Exposure time: 150 seconds



Immunocytochemistry/ Immunofluorescence - Anti-alpha Tubulin (acetyl K40) antibody [6-11B-1] (ab24610)

This image is courtesy of an Abreview submitted by Aaron Halpen

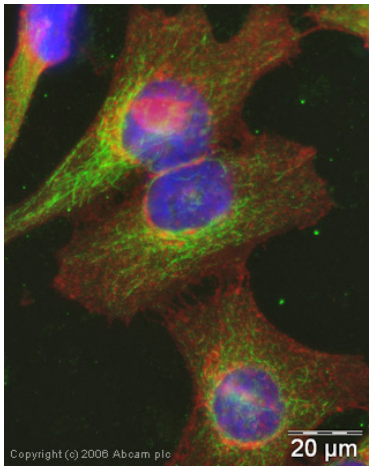
ab24610 staining Acetylated alpha Tubulin in monkey kidney cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 3% PFA + 0.1% GA and blocked with 3% BSA + 0.5% Triton X-100 for 45 minutes at 25°C. Samples were incubated with primary antibody (1/100 in 3% BSA + 0.5% Triton X-100) for 1 hour at 21°C. An Alexa Fluor® 647-conjugated donkey anti-rabbit IgG polyclonal (2 µg/ml) was used as the secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha Tubulin (acetyl K40) antibody [6-11B-1] (ab24610)

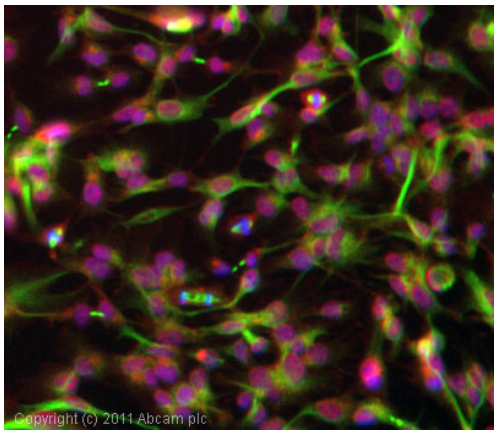
This image is courtesy of an anonymous Abreview

ab24610 at 1/100 dilution staining acetylated alpha tubulinin in prostate carcinoma by immunohistochemistry (formalin/PFA-fixed paraffin-embedded sections). Sections were paraformaldehyde fixed, permeabilized in Triton X-100 prior to blocking in 1% serum for 1 hour at 27°C and then incubated with ab24610 for 12 hours at 4°C. Alexa Fluor® 546 donkey polyclonal to mouse Ig, diluted 1/500, was used as the secondary antibody.



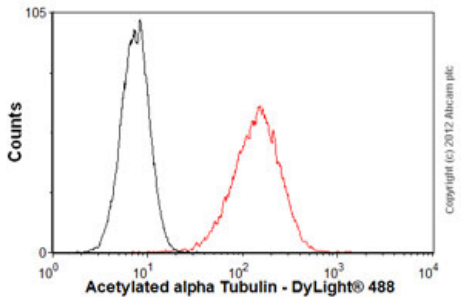
Immunocytochemistry/ Immunofluorescence - Anti-Acetylated alpha Tubulin antibody [6-11B-1] (ab24610)

ICC/IF image of ab24610 stained human HeLa cells. The cells were methanol fixed (5 min) and incubated with the antibody (ab24610, 1µg/ml) for 1h at room temperature. The secondary antibody (green) was Alexa Fluor® 488 goat anti-mouse IgG (H+L) used at a 1/1000 dilution for 1h. Image-iT™ FX Signal Enhancer was used as the primary blocking agent, 5% BSA (in TBS-T) was used for all other blocking steps. DAPI was used to stain the cell nuclei (blue). Alexa Fluor® 594 WGA was used to label plasma membranes (red).



Immunocytochemistry/ Immunofluorescence - Anti-Acetylated alpha Tubulin antibody [6-11B-1] (ab24610)

ICC/IF image of ab24610 stained HepG2 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 (ab24610, 5µg/ml) overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti-mouse IgG - H&L, pre-adsorbed (ab96879) used at a 1/250 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.



Flow Cytometry - Anti-Acetylated alpha Tubulin antibody [6-11B-1] (ab24610)

Overlay histogram showing HeLa cells stained with ab24610 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab24610, 1µg/1x10^6 cells) for 30 min at 22ºC. (This data was generated from a purified version of the antibody. Some lots are produced as ascites fluid. We suggest 1µl/1x10^6 cells for ascites preparations). The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was mouse IgG2b [PLPV219] (ab91366, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HeLa cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

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