abcam

Product datasheet

Anti-alpha Tubulin antibody ab18251

20 Abreviews 161 References 13 Images

Overview

Product name	Anti-alpha Tubulin antibody
Description	Rabbit polyclonal to alpha Tubulin
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, IHC-P, Flow Cyt, WB
Species reactivity	**Reacts with:** Mouse, Rat, Chicken, Cow, Human, Drosophila melanogaster, Indian muntjac, African green monkey, Chinese hamster
Immunogen	Synthetic peptide conjugated to KLH derived from within residues 400 to the C-terminus of Human alpha Tubulin. Read Abcam's proprietary immunogen policy
Positive control	WB: HeLa, HEK-293, HepG2, Caco-2, NIH/3T3 and PC-12 whole cell lysates. ICC/IF: HeLa, Caco-2, NIH/3T3 and 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	Preservative: 0.02% Sodium Azide Constituents: 1% BSA, PBS, pH 7.4
Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab18251** 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
ICC/IF	★★★★★	Use a concentration of 1 - 5 µg/ml.

Application	Abreviews	Notes
IHC-P	★★★★☆	1/2500. Perform heat mediated antigen retrieval via the pressure cooker method before commencing with IHC staining protocol.
Flow Cyt		Use 0.01µg for 10^6 cells.
WB	★★★★★	Use a concentration of 0.5 µg/ml. Detects a band of approximately 50 kDa (predicted molecular weight: 50 kDa). Abcam recommends using milk as the blocking agent.

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.

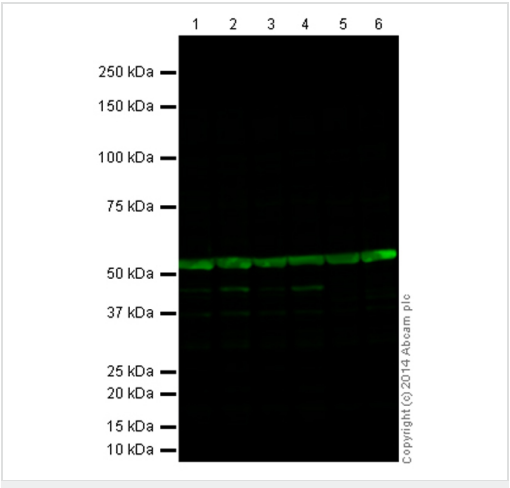
Post-translational modifications

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 (ab18251)

All lanes : Anti-alpha Tubulin antibody (ab18251) at 0.5 µg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) whole cell lysate

Lane 2 : HEK-293 (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 : NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate

Lane 6 : PC-12 (Rat adrenal pheochromocytoma cell line) whole

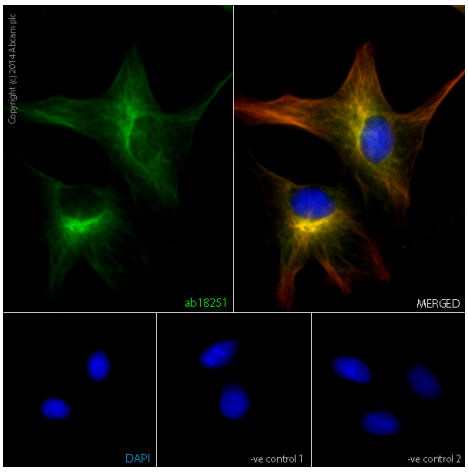
cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (Alexa Fluor® 790) (ab175781) at 1/10000 dilution

Predicted band size: 50 kDa
Observed band size: 52 kDa
why is the actual band size different from the predicted?

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 ab18251 overnight at 4°C. Antibody binding was detected using Anti-Rabbit Alexa Fluor® 790 (ab175781) at a 1:10,000 dilution for 1hr at room temperature and then imaged using the Licor Odyssey CLx.

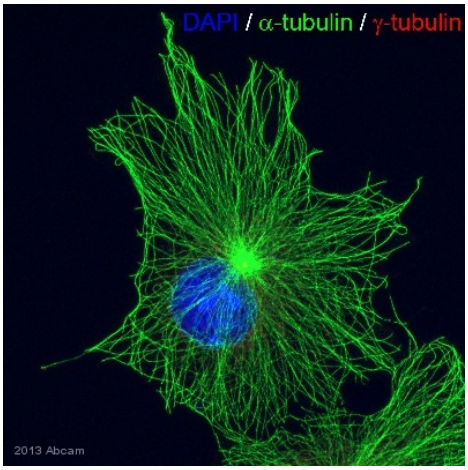


Immunocytochemistry/ Immunofluorescence - Anti-alpha Tubulin antibody (ab18251)

ab18251 staining alpha-Tubulin in SV40LT-SMC cells.

The cells were fixed with 100% methanol for 5 minutes and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1 hour. The cells were then incubated with ab18251 at 1 µl/ml and ab7291 at 1 µg/ml overnight at +4°C, followed by a further incubation at room temperature for 1 hour with an anti-rabbit AlexaFluor® 488 (ab150081) at 2 µg/ml (shown in green) and anti-mouse AlexaFluor® 594 (ab150120) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labeled 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.

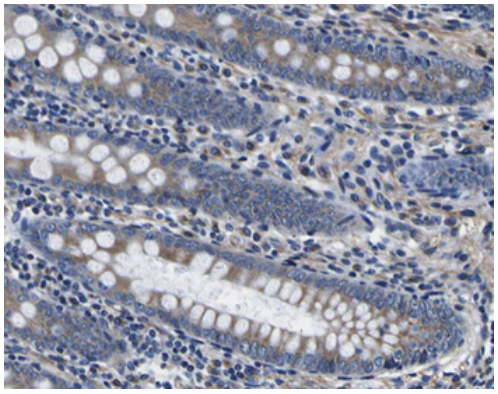


Immunocytochemistry/ Immunofluorescence - Anti-alpha Tubulin antibody (ab18251)

This image is courtesy of an anonymous Abreview

ab18251 staining alpha Tubulin in the COS-7 (African green monkey kidney fibroblast-like cell line) cells by ICC/IF (Immunocytochemistry/immunofluorescence).

Cells were fixed with methanol and blocked with 5% BSA for 60 minutes at 21°C. Samples were incubated with primary antibody (1/500) for 17 hours at 4°C. An Alexa Fluor®488-conjugated Goat anti-rabbit IgG polyclonal(1/400) was used as the secondary antibody.

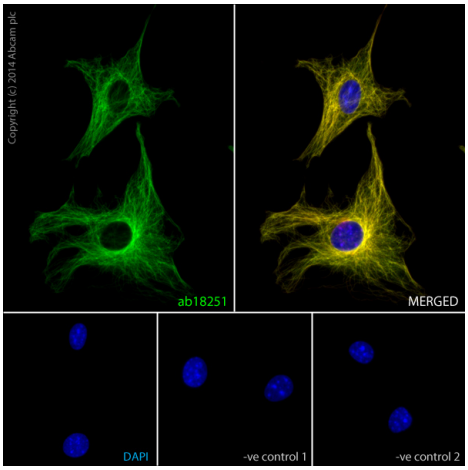


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha Tubulin antibody (ab18251)

Image courtesy of Human Protein Atlas

ab18251 staining alpha Tubulin. Paraffin embedded human appendix tissue was incubated with ab18251 (1/2500 dilution) for 30 mins at room temperature. Antigen retrieval was performed by heat induction in citrate buffer pH 6. ab18251 was tested in a tissue microarray (TMA) containing a wide range of normal and cancer tissues as well as a cell microarray consisting of a range of commonly used, well characterised human cell lines.

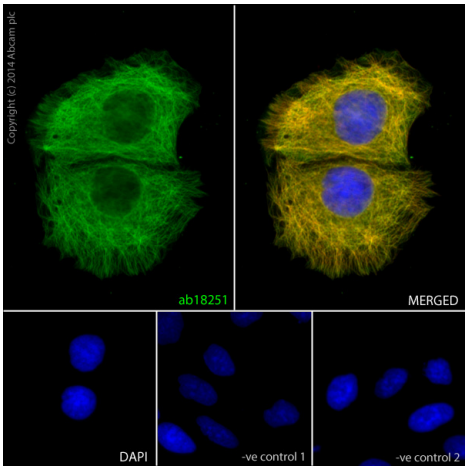
Further results for this antibody can be found at www.proteinatlas.org



Immunocytochemistry/ Immunofluorescence - Anti-alpha Tubulin antibody (ab18251)

ab18251 staining alpha-Tubulin in NIH3T3 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 ab18251 at 1µl/ml and ab7291 at 1µg/ml overnight at +4°C, followed by a further incubation at room temperature for 1h with an anti-rabbit AlexaFluor® 488 (ab150081) at 2 µg/ml (shown in green) and anti-mouse AlexaFluor® 594 (ab150120) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labelled in blue with DAPI.

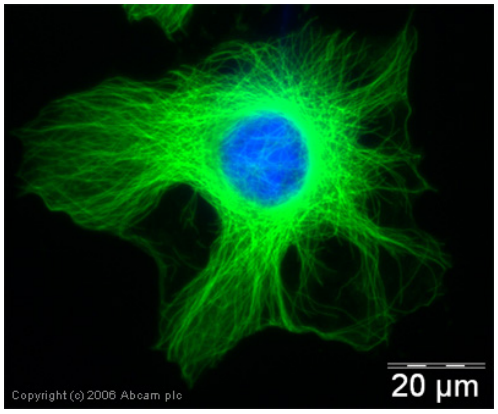
Negative controls: 1– Rabbit primary antibody and anti-mouse secondary antibody; 2 – Mouse primary antibody and anti-rabbit secondary antibody. Controls 1 and 2 indicate that there is no unspecific reaction between primary and secondary antibodies used.



Immunocytochemistry/ Immunofluorescence - Anti-alpha Tubulin antibody (ab18251)

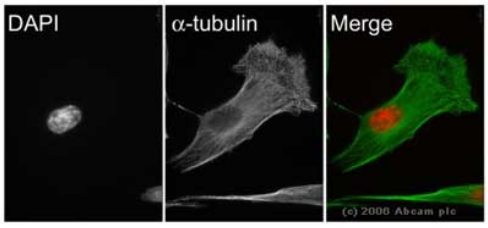
ab18251 staining alpha-Tubulin in Caco-2 cells. The cells were fixed with 100% methanol (5min) and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with ab18251 at 5µl/ml and ab7291 at 1µg/ml overnight at +4°C, followed by a further incubation at room temperature for 1h with an anti-rabbit AlexaFluor® 488 (ab150081) at 2 µg/ml (shown in green) and anti-mouse AlexaFluor® 594 (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 (ab18251)

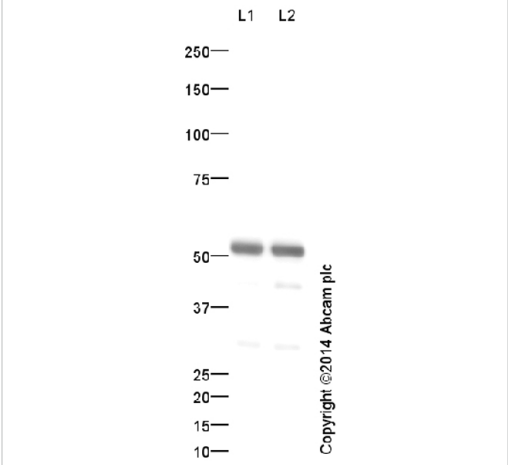
ICC/IF image of ab18251 stained human HeLa cells. The cells were methanol fixed (5 min) and incubated with the antibody (ab18251, 1µg/ml) for 1h at room temperature. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Image-iT^{TM} FX Signal 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).



Immunocytochemistry/ Immunofluorescence - Anti-alpha Tubulin antibody (ab18251)

ab18251 at a 1/8000 dilution staining human HeLa cells by immunocytochemistry. The cells were paraformaldehyde fixed and incubated with the antibody for 30 minutes. The secondary antibody was a Cy3 conjugated Goat Anti-Rabbit IgG (H+L). The image shows staining of an interphase IM cell.

This image is courtesy of an Abreview by **Kirk McManus** submitted on **27 February 2006.**



Western blot - Anti-alpha Tubulin antibody (ab18251)

All lanes : Anti-alpha Tubulin antibody (ab18251) at 1 µg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2 : A431 (Human epithelial carcinoma cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Developed using the ECL technique.

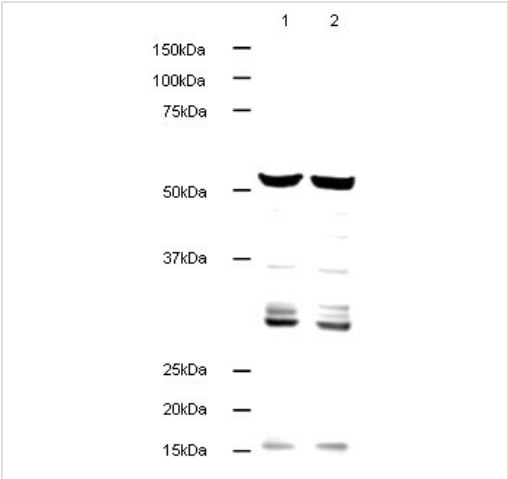
Performed under reducing conditions.

Predicted band size: 50 kDa

Observed band size: 50 kDa

Exposure time: 1 minute

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 2% Bovine Serum Albumin before being incubated with ab18251 overnight at 4°C. Antibody binding was detected using an anti-rabbit HRP (ab97051), and visualised using ECL development solution ab133406



Western blot - Anti-alpha Tubulin antibody (ab18251)

All lanes : Anti-alpha Tubulin antibody (ab18251) at 0.5 µg/ml

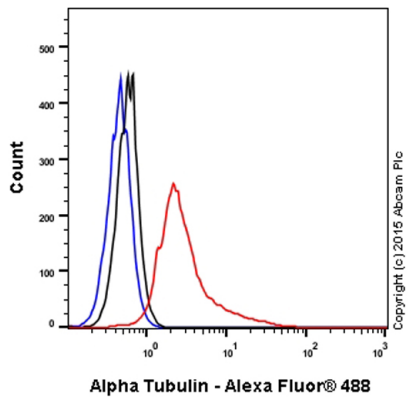
Lane 1 : HeLa lysate
Lane 2 : A431 lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Alexa Flour Goat polyclonal to Rabbit IgG (700) at 1/10000 dilution

Predicted band size: 50 kDa
Observed band size: 50 kDa
Additional bands at: 30 kDa (possible cross reactivity)

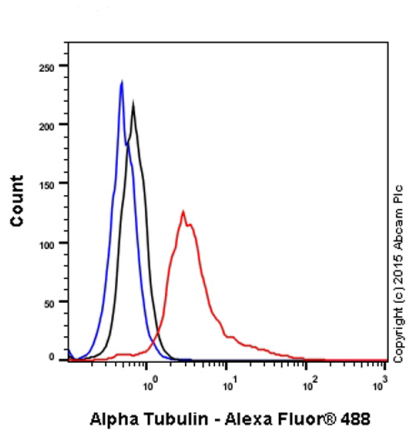
ab18251 detects a strong band at 50 kDa corresponding to alpha tubulin. Cross-reactivity is also seen with other lower molecular weight bands. This may be reduced by using the antibody at a lower working concentration.



Flow Cytometry - Anti-alpha Tubulin antibody (ab18251)

Overlay histogram showing NIH3T3 cells stained with ab18251 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 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 (ab18251, 0.01µg/1x10^6 cells) for 30 min at 22ºC. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150081) at 1/4000 dilution for 30 min at 22ºC. Isotype control antibody (black line) was rabbit IgG (polyclonal) (ab27478, 0.01µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control.

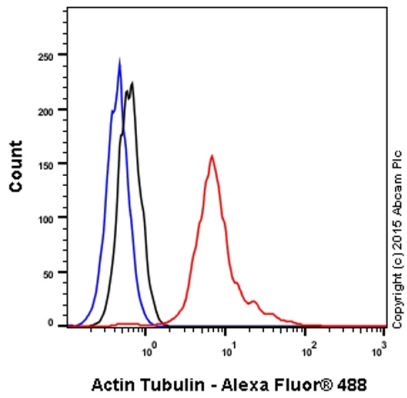
Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



Flow Cytometry - Anti-alpha Tubulin antibody (ab18251)

Overlay histogram showing SV40LT-SMC cells stained with ab18251 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 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 (ab18251, 0.01µg/1x10^6 cells) for 30 min at 22ºC. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150081) at 1/4000 dilution for 30 min at 22ºC. Isotype control antibody (black line) was rabbit IgG (polyclonal) (ab27478, 0.01µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



Flow Cytometry - Anti-alpha Tubulin antibody (ab18251)

Overlay histogram showing Caco2 cells stained with ab18251 (red line). The cells were fixed with 80% methanol (5 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 (ab18251, 0.01µg/1x10^6 cells) for 30 min at 22ºC. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150081) at 1/4000 dilution for 30 min at 22ºC. Isotype control antibody (black line) was rabbit IgG (polyclonal) (ab27478, 0.01µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors