abcam

Product datasheet

Anti-alpha Tubulin antibody - Microtubule Marker ab18251

21 Abreviews 331 References 11 Images

Overview

Product name	Anti-alpha Tubulin antibody - Microtubule Marker
Description	Rabbit polyclonal to alpha Tubulin - Microtubule Marker
Host species	Rabbit
Specificity	Replenishment batches of our polyclonal antibody, ab18251 are tested in WB. Previous batches were additionally validated in Flow Cyt and ICC/IF. These applications are still expected to work and are covered by our Abpromise guarantee. You may also be interested in our alternative recombinant antibody, **ab52866**.
Tested applications	**Suitable for:** ICC/IF, Flow Cyt (Intra), WB
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Chicken, Cow, 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.
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

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

Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.

Purity Immunogen affinity purified

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee 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	★★★★★ (12)	Use a concentration of 1 - 5 µg/ml.
Flow Cyt (Intra)		Use 0.01µg for 10^6 cells.
WB	★★★★★ (6)	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

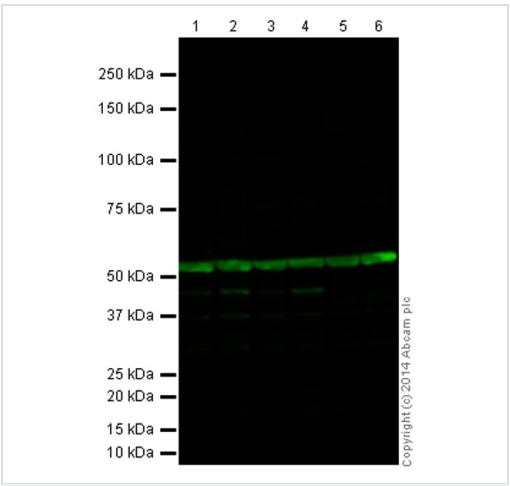
Function Tubulin is the major constituent of microtubules. It binds two moles of GTP, one at an exchangeable site on the beta chain and one at a non-exchangeable site on the alpha chain.

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 - Microtubule Marker (ab18251)

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

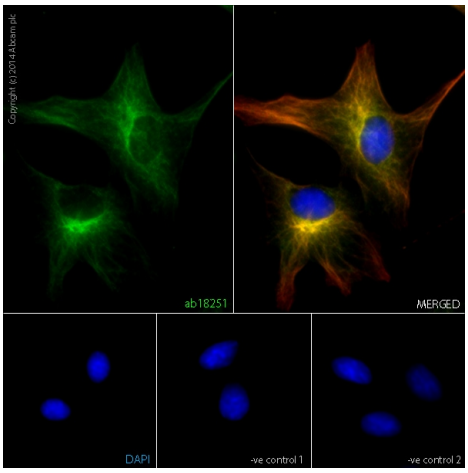
Lane 1 : HeLa (Human epithelial carcinoma cell line) whole cell lysate
Lane 2 : HEK-293 (Human embryonic kidney cell line) whole cell lysate
Lane 3 : HepG2 (Human hepatocellular liver carcinoma cell line) whole cell lysate
Lane 4 : Caco-2 (Human colonic carcinoma cell line) whole cell lysate
Lane 5 : 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

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.

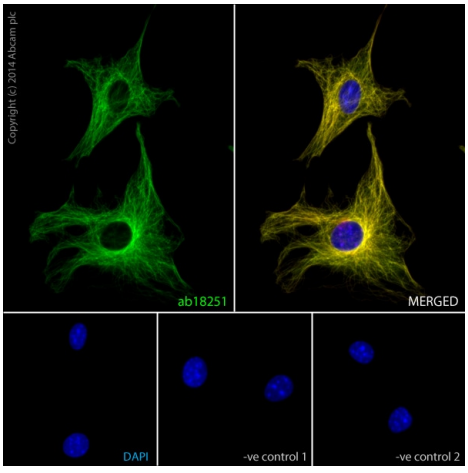


Immunocytochemistry/ Immunofluorescence - Anti-alpha Tubulin antibody - Microtubule Marker (ab18251)

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 Alexa Fluor® 488 (**ab150081**) at 2 µg/ml (shown in green) and anti-mouse Alexa Fluor® 594 (**ab150120**) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labeled in blue with DAPI.

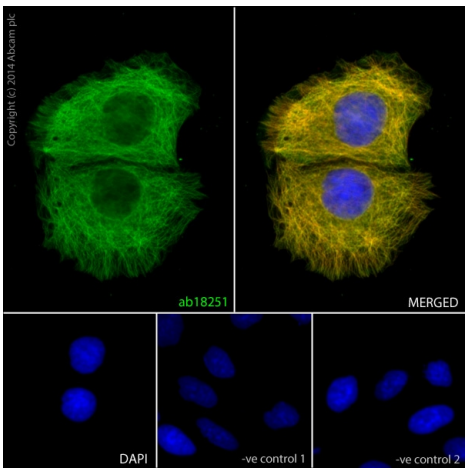
Negative controls: 1– Rabbit primary antibody and anti-mouse secondary antibody; 2 – Mouse primary antibody and anti-rabbit secondary antibody. Controls 1 and 2 indicate that there is no unspecific reaction between primary and secondary antibodies used.



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

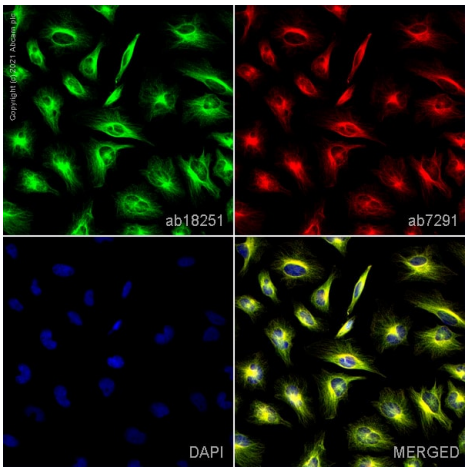
Negative controls: 1– Rabbit primary antibody and anti-mouse secondary antibody; 2 – Mouse primary antibody and anti-rabbit secondary antibody. Controls 1 and 2 indicate that there is no unspecific reaction between primary and secondary antibodies used.



Immunocytochemistry/ Immunofluorescence - Anti-alpha Tubulin antibody - Microtubule Marker (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 5 µl/ml and **ab7291** at 1 µg/ml overnight at +4°C, followed by a further incubation at room temperature for 1h with an anti-rabbit Alexa Fluor® 488 (**ab150081**) at 2 µg/ml (shown in green) and anti-mouse Alexa Fluor® 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.

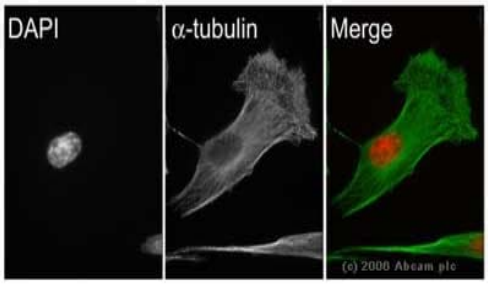


Immunocytochemistry/ Immunofluorescence - Anti-alpha Tubulin antibody - Microtubule Marker (ab18251)

ab18251 staining alpha Tubulin in HeLa cells. The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% PBS-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 ab18251 at 1µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Also suitable in cells fixed with 100% methanol (5 min).

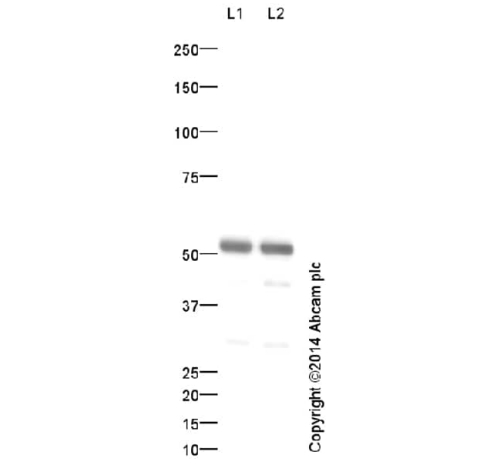
Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



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

All lanes : Anti-alpha Tubulin antibody - Microtubule Marker (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.

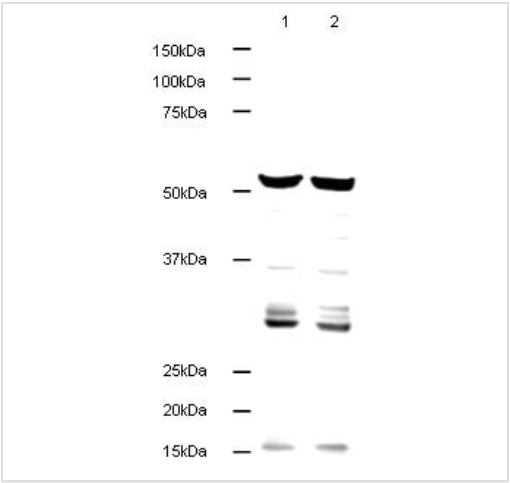
Performed under reducing conditions.

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 - Microtubule Marker (ab18251)

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

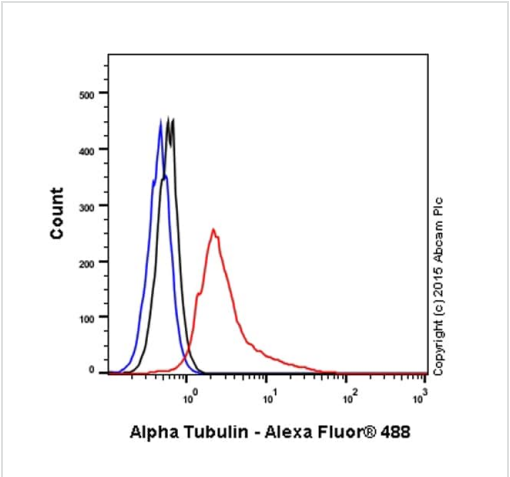
Lane 1 : HeLa lysate
Lane 2 : A431 lysate

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)

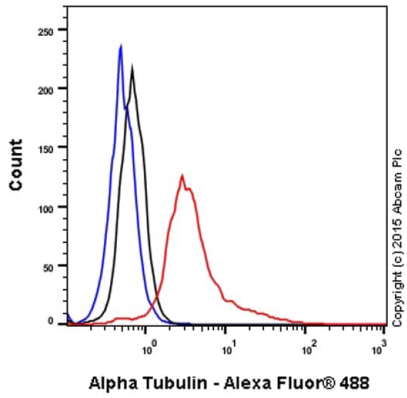
ab18251 detects a strong band at 50 kDa corresponding to alpha tubulin. Cross-reactivity is also seen with other lower molecular weight bands. This may be reduced by using the antibody at a lower working concentration.



Flow Cytometry (Intracellular) - Anti-alpha Tubulin antibody - Microtubule Marker (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 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (**ab150081**) at 1/4000 dilution for 30 min at 22°C. 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 (Intracellular) - Anti-alpha Tubulin antibody - Microtubule Marker (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 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (**ab150081**) at 1/4000 dilution for 30 min at 22°C. 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.

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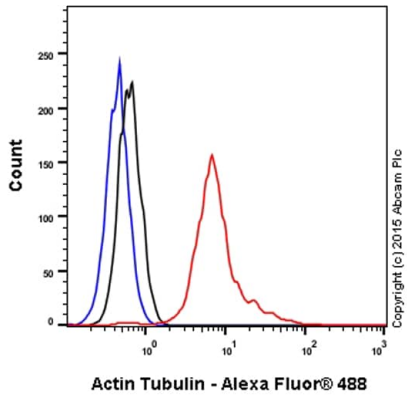

Flow Cytometry (Intracellular) - Anti-alpha Tubulin antibody - Microtubule Marker (ab18251)

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 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (**ab150081**) at 1/4000 dilution for 30 min at 22°C. 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.

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