abcam

Product datasheet

Anti-Tubulin antibody [YOL1/34] - Microtubule Marker ab6161

21 Abreviews 145 References 10 Images

Overview

Product name	Anti-Tubulin antibody [YOL1/34] - Microtubule Marker
Description	Rat monoclonal [YOL1/34] to Tubulin - Microtubule Marker
Host species	Rat
Tested applications	**Suitable for:** WB, ICC/IF, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Dog, Saccharomyces cerevisiae, Plants, Schizosaccharomyces pombe, a wide range of other species, Mammals, Alligator
Immunogen	Full length native protein (purified) corresponding to Saccharomyces cerevisiae Tubulin.
Epitope	ab6161 binds to an epitope between amino acids 414 and 422 of alpha tubulin.
Positive control	WB: HeLa and NIH3T3 whole cell lysates and rat brain tissue lysate. Flow Cyt (Intra): methanol fixed/tween permeabilised HeLa cells. ICC/IF: HeLa, NIH/3T3 and human macrophage cells.
General notes	We can conjugate this antibody to FITC for you (please see **ab150252** for details). This antibody can be used as a Western blotting loading control (Kops et al.) and as a Microtubule Marker. Has been used for the selection of specific recombinant antibodies engineered to incorporate its epitope. It is also useful for studying the function of microtubules. This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**. 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 Constituents: PBS, 6.97% L-Arginine
Purity	Protein G purified
Primary antibody notes	Has been used for the selection of specific recombinant antibodies engineered to incorporate its epitope. It is also useful for studying the function of microtubules.
Clonality	Monoclonal
Clone number	YOL1/34
Isotype	IgG2a

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab6161 in the following tested applications.

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

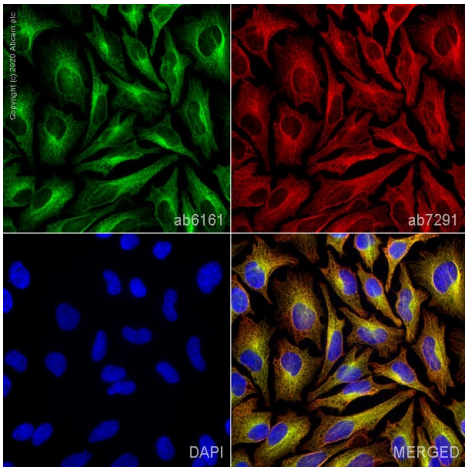
Application	Abreviews	Notes
WB	★★★★★ **(10)**	Use a concentration of 1 µg/ml.
ICC/IF	★★★★★ **(11)**	Use a concentration of 5 µg/ml.
Flow Cyt (Intra)		Use 1µg for 10^6 cells. **ab18450** - Rat monoclonal IgG2a, is suitable for use as an isotype control with this antibody.

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	Undergoes a tyrosination/detyrosination cycle, the cyclic removal and re-addition of a C-terminal tyrosine residue by the enzymes tubulin tyrosine carboxypeptidase (TTCP) and tubulin tyrosine ligase (TTL), respectively. 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-tubulins 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.

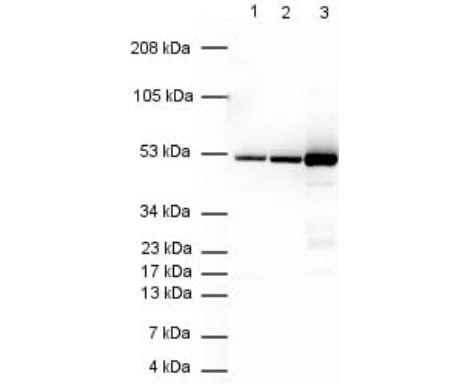
Images



Immunocytochemistry/ Immunofluorescence - Anti-Tubulin antibody [YOL1/34] - Microtubule Marker (ab6161)

ab6161 staining 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 ab6161 at 5µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with **ab150165**, Goat polyclonal Secondary Antibody to Rat 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).

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



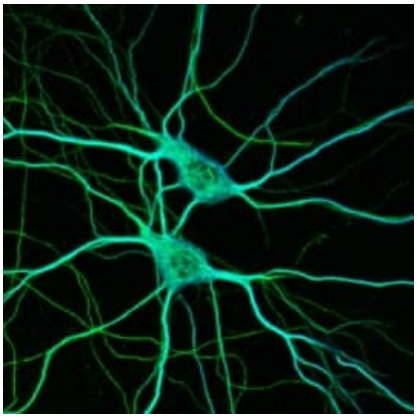
Western blot - Anti-Tubulin antibody [YOL1/34] - Microtubule Marker (ab6161)

Western blot against tubulin with ab6161 at 1/3000. Secondary Rabbit anti-Rat IgG HRP (**ab6734**)was used at 1/2000. Exposure time: 2mins.

Lane 1: 20µg/lane HeLa (Human) whole cell lysates (**ab7898**).

Lane 2: 20µg/lane 3T3 (Mouse) whole cell lysate (**ab7901**).

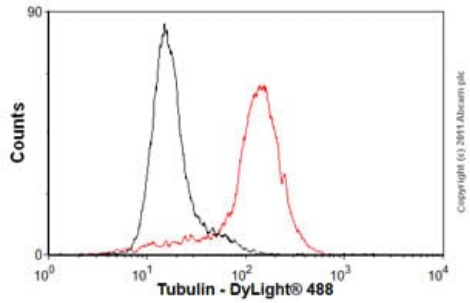
Lane 3: 20µg/lane Rat brain tissue lysate (**ab7942**).



Immunocytochemistry/ Immunofluorescence - Anti-Tubulin antibody [YOL1/34] - Microtubule Marker (ab6161)

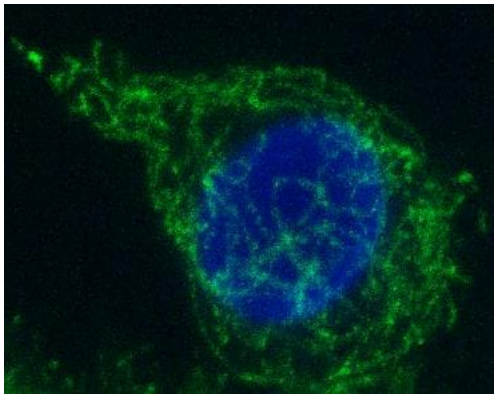
Confocal image of 21 day in vitro rat hippocampal neurons, stained with rat monoclonal antibody to Tubulin - Microtubule Marker (ab6161) in green at 1/500 and Microtubule Associated protein 2 in blue.

This picture was kindly supplied as part of the review submitted by Dr Jonathon Burman.



Flow Cytometry (Intracellular) - Anti-Tubulin antibody [YOL1/34] - Microtubule Marker (ab6161)

Overlay histogram showing HeLa cells stained with ab6161 (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 (ab6161, 1µg/1x10^6 cells) for 30 min at 22ºC. The secondary antibody used was DyLight® 488 goat anti-rat IgG (H+L) (**ab98386**) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was rat IgG2a [aRTK2758] (**ab18450**, 1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.

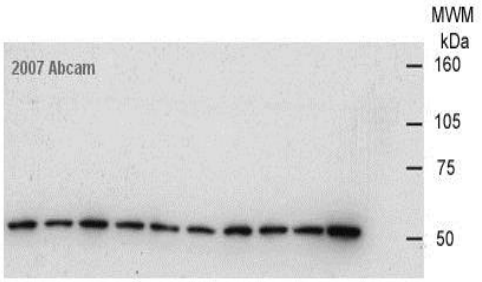


Immunocytochemistry/ Immunofluorescence - Anti-Tubulin antibody [YOL1/34] - Microtubule Marker (ab6161)

Cultured human macrophages were used with ab6161 at 1/1000 for immunofluorescence. Cells were fixed with cold 2% formaldehyde for 20mins.

Green staining is Alexa 568, Blue staining is DAPI stain.

This cell represents a young macrophage, the staining patterns varied as the cells aged in culture.



Western blot - Anti-Tubulin antibody [YOL1/34] - Microtubule Marker (ab6161)

This image is courtesy of an anonymous Abreview

All lanes : Anti-Tubulin antibody [YOL1/34] - Microtubule Marker (ab6161) at 1/2000 dilution

All lanes : Yeast (Saccharomyces cerevisiae) whole cell extract prepared by bead-beating

Lysates/proteins at 5 µg per lane.

Secondary

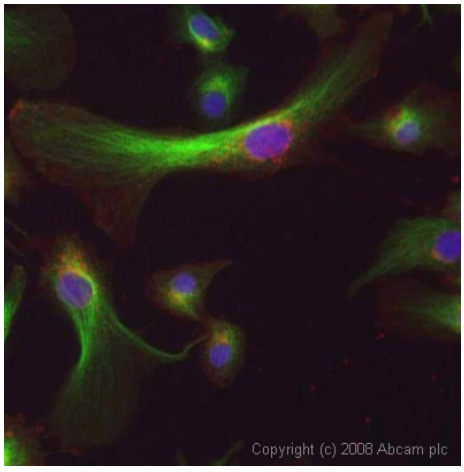
All lanes : HRP conjugated goat anti-rat antibody

Developed using the ECL technique.

Performed under reducing conditions.

Observed band size: 50 kDa

Exposure time: 30 seconds



Immunocytochemistry/ Immunofluorescence - Anti-Tubulin antibody [YOL1/34] - Microtubule Marker (ab6161)

ICC/IF image of ab6161 stained human HepG2 cells. The cells were 4% PFA fixed (10 min), permabilised in 0.1% PBS-Tween (20 min) and incubated with the antibody (ab6161, 1µg/ml) for 1h at room temperature. 1%BSA / 10% normal goat serum / 0.3M glycine was used to block non-specific protein-protein interactions. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rat IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red). DAPI was used to stain the cell nuclei (blue). This antibody also gave a positive IF result in HeLa, HEK 293 and MCF7 cells.

Western blot - Anti-Tubulin antibody [YOL1/34] - Microtubule Marker (ab6161)

Anti-Tubulin antibody [YOL1/34] - Microtubule Marker (ab6161) at 1 µg/ml + Brain (Rat) Tissue Lysate at 10 µg

Secondary

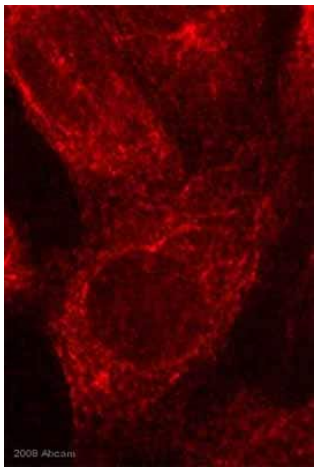
Rabbit polyclonal to Rat IgG - H&L (HRP) at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Observed band size: 54 kDa

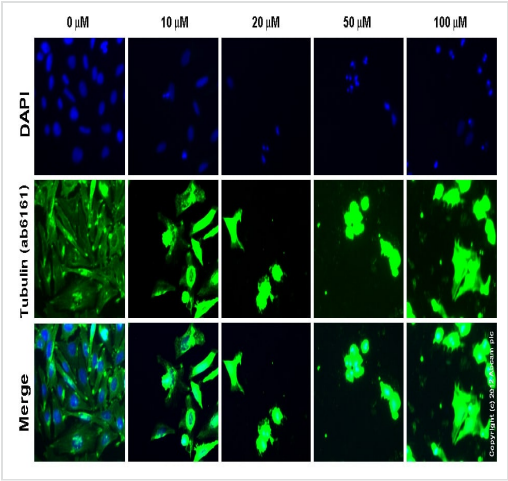
Exposure time: 3 minutes



Immunocytochemistry/ Immunofluorescence - Anti-Tubulin antibody [YOL1/34] - Microtubule Marker (ab6161)

This image is courtesy of an anonymous Abreview

ab6161 staining mouse NIH 3T3 fibroblast cells by ICC/IF. Cells were PFA fixed and permeabilized in 0.2% Triton X-100 prior to blocking in 5% BSA for 45 minutes at RT. The primary antibody was diluted 1/1000 and incubated with the sample for 1 hour. An Alexa Fluor® 568 conjugated goat anti-rat antibody, diluted 1/3000, was used as the secondary.



Immunocytochemistry/ Immunofluorescence - Anti-Tubulin antibody [YOL1/34] - Microtubule Marker (ab6161)

ab6161 staining tubulin HeLa cells treated with anisomycin (**ab120495**), by ICC/IF. Increase in tubulin expression correlates with increased concentration of anisomycin as described in literature.

The cells were incubated at 37°C for 6h in media containing different concentrations of **ab120495** (anisomycin) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab6161 (5 µg/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 goat anti-rat polyclonal antibody (**ab98386**) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.

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