


Anti-Tubulin antibody [YL1/2] - Loading Control ab6160

★★★★★ [38 Abreviews](#) [500 References](#) [6 Images](#)

Overview

Product name	Anti-Tubulin antibody [YL1/2] - Loading Control
Description	Rat monoclonal [YL1/2] to Tubulin - Loading Control
Host species	Rat
Tested applications	Suitable for: WB, ICC/IF, IHC-P, Flow Cyt (Intra)
Species reactivity	<p>Reacts with: Mouse, Rat, Human</p> <p>Predicted to work with: Pig, Saccharomyces cerevisiae, Xenopus laevis, Caenorhabditis elegans, Drosophila melanogaster, Schizosaccharomyces pombe, a wide range of other species, Mammals, African green monkey </p>
Immunogen	Full length native protein (purified) corresponding to Saccharomyces cerevisiae Tubulin.
Epitope	The YL1/2 monoclonal epitope has been mapped to the last 8 residues (GEEEGEEY) at the carboxy terminus of alpha tubulin when tyrosinated (PubMed IDs: 6415068, 6204858).
Positive control	ICC/IF: HeLa cells. IHC-P: Human colon tissue. WB: HeLa, NIH/3T3, BALB/3T3 and PC-12 whole cell lysate. Flow Cyt (Intra): HeLa cells.
General notes	<p>This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact orders@abcam.com.</p> <p>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.</p> <p>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</p>

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	<p>pH: 7.40</p> <p>Preservative: 0.02% Sodium azide</p> <p>Constituents: PBS, 6.97% L-Arginine</p>

Purity	Protein G purified
Primary antibody notes	This antibody can be used as a loading control on Western blots (Allen <i>et al.</i>) and is not detected by anti-mouse Ig secondaries. It has been used in epitope tagging procedures to detect proteins tagged with a C-terminal Gly-Gly-Phe(OH) epitope. Under some circumstances this antibody may cross-react with other protein including <i>E. coli</i> rec A and oxidized actin.
Clonality	Monoclonal
Clone number	YL1/2
Isotype	IgG2a

Applications

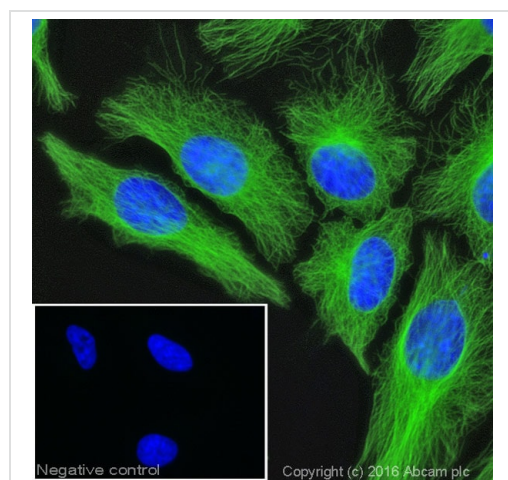
The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab6160 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★★ (17)	1/5000 - 1/10000.
ICC/IF	★★★★★ (14)	1/1000. (see PMID: 16230461)
IHC-P	★★★★★ (2)	Use at an assay dependent concentration.
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	<p>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.</p> <p>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 TTL10 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 polyglutamylated, and reciprocally. The precise function of such modifications is still unclear but they regulate the assembly and dynamics of axonemal microtubules.</p> <p>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.</p>
Cellular localization	Cytoplasm > cytoskeleton.

Images



Immunocytochemistry/ Immunofluorescence - Anti-Tubulin antibody [YL1/2] - Loading Control (ab6160)

ICC/IF image of ab6160 stained HeLa (Human epithelial cell line from cervix adenocarcinoma) cells.

The cells were fixed in 100% methanol 5 minutes, permeabilized with 0.1% Triton X-100 for 5 minutes and then incubated in 1% BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1 hour to block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab6160, 1/1000 dilution) overnight at +4°C. The secondary antibody (green) was **ab150165** Alexa Fluor® 488 goat anti-rat IgG (H+L) pre-adsorbed, used at a 1/1000 dilution for 1 hour. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

The negative control (inset) is a secondary-only assay to demonstrate low non-specific binding of the secondary antibody.

This product also gave a positive signal under the same testing conditions in HeLa cells fixed with 4% formaldehyde (10 minutes).



Western blot - Anti-Tubulin antibody [YL1/2] (ab6160)

Lanes 1 & 3 : Anti-Tubulin antibody [YL1/2] - Loading Control (ab6160) at 1/5000 dilution

Lanes 2 & 4 : Anti-Tubulin antibody [YL1/2] - Loading Control (ab6160) at 1/10000 dilution

Lanes 1-2 : HeLa (Human epithelial carcinoma cell line) whole cell lysate

Lanes 3-4 : BALB/3T3 whole cell lysate (**ab7901**)

Lysates/proteins at 20 µg per lane.

Secondary

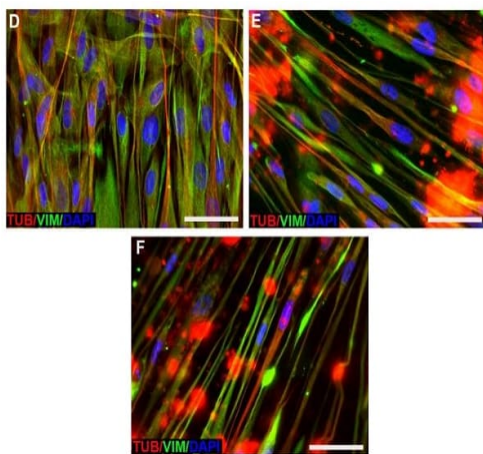
All lanes : Rabbit Anti-Rat IgG H&L (HRP) (**ab6734**) at 1/2000 dilution

Predicted band size: 50 kDa

Observed band size: 52 kDa

Additional bands at: 17 kDa, 34 kDa, 80 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 10 seconds



Immunocytochemistry/ Immunofluorescence - Anti-Tubulin antibody [YL1/2] - Loading Control (ab6160)

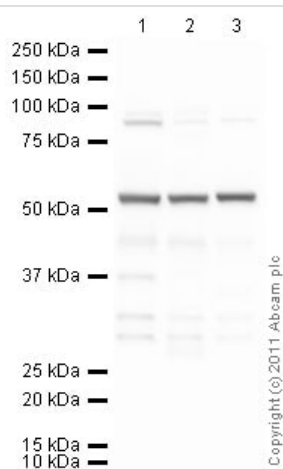
Loison-Robert et al PLoS One. 2018 Jan 25;13(1):e0190014. doi: 10.1371/journal.pone.0190014. eCollection 2018. Fig 5. Reproduced under the Creative Commons license <http://creativecommons.org/licenses/by/4.0/>

Cytoskeleton and major extracellular matrix proteins in human DPSC (Dental pulp stem cell) were analyzed by immunofluorescence.

Vimentin and tubulin (Panel D, control, E, (BD) and F, (BR)) are shown.

After 7 days in contact with/without the materials (Biodentine (BD) and Bioroot (BR)), coated coverslip cultures were fixed in PBS (pH 7.4) containing 4% paraformaldehyde/5% sucrose for 10 minutes. For detection of intracellular molecules, the cells on the coverslips were permeabilized using 0.5% Triton X-100. To block background staining, cells were treated with PBS containing 1% BSA/1% glycine at 37°C for 20 minutes. Samples were incubated with the primary antibody at 4°C overnight or at 37°C for 2 hours. For double immunostaining, primary antibodies were incubated as above. Samples were then incubated with the appropriate secondary antibodies at 37°C for 1 hour. Cell nuclei were stained using DAPI.

Scale Bar: 100 µm.



Western blot - Anti-Tubulin antibody [YL1/2] - Loading Control (ab6160)

All lanes : Anti-Tubulin antibody [YL 1/2] - Loading Control (ab6160) 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 : PC-12 (Rat adrenal pheochromocytoma cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Peroxidase Conjugated Rabbit Anti-Rat IgG (H+L) at 1/10000 dilution

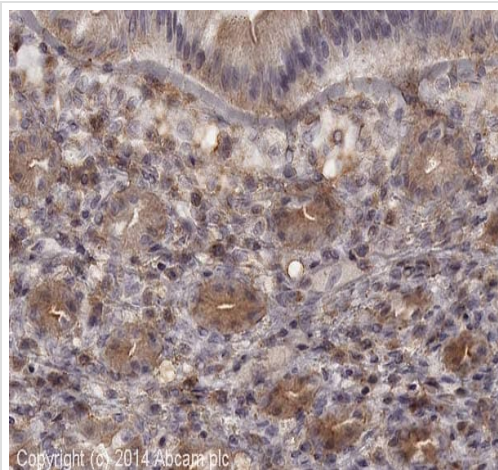
Performed under reducing conditions.

Predicted band size: 50 kDa

Observed band size: 52 kDa

Additional bands at: 85 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 8 minutes



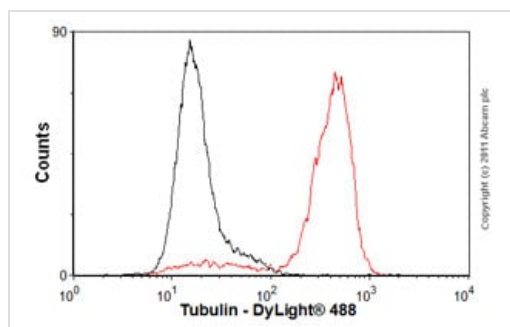
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Tubulin antibody [YL1/2] - Loading Control (ab6160)

IHC image of Tubulin staining in human colon formalin fixed paraffin embedded tissue section*, performed on a Leica Bond™ system using the standard protocol F.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer pH 6 for 20 minutes. The section was then incubated with ab6160, 5 µg/ml, for 15 minutes at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



Flow Cytometry (Intracellular) - Anti-Tubulin antibody [YL1/2] - Loading Control (ab6160)

Overlay histogram showing HeLa (Human epithelial cell line from cervix adenocarcinoma) cells stained with ab6160 (red line).

The cells were fixed with 80% methanol (5 minutes) and then permeabilized with 0.1% PBS-Tween for 20 minutes. 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 (ab6160, 1 µg/1x10⁶ cells) for 30 minutes at 22°C. The secondary antibody used was DyLight® 488 goat anti-rat IgG (H+L) (**ab98386**) at 1/500 dilution for 30 minutes at 22°C. Isotype control antibody (black line) was rat IgG2a [aRTK2758] (**ab18450**, 1 µg/1x10⁶ cells) used under the same conditions.

Acquisition of >5,000 events was performed.

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