Product datasheet

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

35 Abreviews 205 References 13 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: ELISA, IHC-Fr, IP, RIA, WB, Flow Cyt, ICC/IF, IHC (PFA fixed), IHC-P, IHC - Wholemount, IHC (Methanol fixed)

Species reactivity: Reacts with: Mouse, Human, Pig, Saccharomyces cerevisiae, Xenopus laevis, Caenorhabditis elegans, Drosophila melanogaster, Schizosaccharomyces pombe, African green monkey

Predicted to work with: a wide range of other species, Mammals

Immunogen: Full length native protein (purified) corresponding to Saccharomyces cerevisiae Tubulin.

Epitope: A linear sequence requiring an aromatic residue at the C terminus, with the two adjacent amino acids being negatively charged (represented by Glu-Glu-Tyr in Tyr-Tubulin).

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: HeLa cells.

General notes: This antibody clone is manufactured by Abcam.

This antibody can be used as a loading control on Western blots (Allen et al.) and is not detected by anti-mouse IgG 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 E. coli recA and oxidized actin.

If you require this antibody in a particular buffer formulation or a particular conjugate for your experiments, please contact orders@abcam.com or you can find further information here.

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: IgG fraction

Primary antibody notes: This antibody can be used as a loading control on Western blots (Allen et al.) and is not detected.
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**Clonality**
Monoclonal

**Clone number**
YL1/2

**Isotype**
IgG2a

**Applications**

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.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tbody>
<tr>
<td>ELISA</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>IHC-Fr</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>IP</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>RIA</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/5000 - 1/10000.</td>
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<tr>
<td>Flow Cyt</td>
<td></td>
<td>Use 1µg for 10^6 cells. ab18450 - Rat monoclonal IgG2a, is suitable for use as an isotype control with this antibody.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/1000. (see PMID: 16230461)</td>
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<tr>
<td>IHC (PFA fixed)</td>
<td></td>
<td>Use a concentration of 5 µg/ml. (from PubMed:16966421)</td>
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<tr>
<td>IHC-P</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>IHC - Wholemount</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration. PubMed: 25368174</td>
</tr>
<tr>
<td>IHC (Methanol fixed)</td>
<td></td>
<td>1/100. (from PubMed:16943269).</td>
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**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 monoglycyalted but not polyglycyalted 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**

**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.
Egr3 localization is associated with microtubule organization in mouse oocytes.

Mouse oocytes stained for Tubulin using ab6160 (Right panels, red) in ICC/IF.

The localization of Egr3 and microtubule at thawing of vitrified oocytes. Vitrified MII oocytes were stored in LN$_2$ for 2 weeks. Oocytes were taken out from LN$_2$, incubated in decreasing concentrations of sucrose, and then fixed immediately. These oocytes were subjected to immunofluorescence staining with anti-Egr3 and ab6160 antibodies. Arrows indicate the growing arrays of microtubules at the site of Egr3 accumulation.

**Green:** Egr3.

**Red:** Microtubule (MT).

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).
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

All lanes: Anti-Tubulin antibody [Y1/2] - 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
why is the actual band size different from the predicted?

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

Exposure time: 8 minutes
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.

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 why is the actual band size different from the predicted?
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] (ab6160)

This image is courtesy of an Abreview submitted by Stephanie Chrysanthou.

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

This image is courtesy of Anonymous Abreview.

ab6160 staining Tubulin in mouse trophoblast giant cells by ICC/IF (Immunocytochemistry/Immunofluorescence).

Cells were fixed with methanol and blocked with 0.5% BSA for 30 minutes at 20°C. Samples were incubated with primary antibody (0.5% BSA, 0.1% Tween-20 PBS) for 1 hour at 20°C. An Alexa Fluor® 568 polyclonal Goat anti-Rat IgG (H+L) Cross-Adsorbed (1/750 dilution) was used as the secondary antibody.

ab6160 staining Tubulin in mouse MEF cells by Immunocytochemistry/Immunofluorescence.

Cells were fixed with 2% PFA and 96% Ethanol. Samples were incubated with primary antibody (1/2000 in 0.1% Saponin/1% BSA/PBS) for 1 hour. A Cy3®-conjugated goat polyclonal to rat IgG (H&L) was used at dilution at 1/500 as secondary antibody.

Red staining in the image represents Tubulin, whereas the green one resembles gamma-tubulin.
ab6160 staining mouse prostate tissue sections by IHC-P.

The tissue was serial sectioned at 6 microns, formaldehyde fixed and subjected to heat mediated antigen retrieval prior to blocking in 3% peroxidase for 5 minutes at 27°C. The primary antibody was diluted 1/500 and incubated with the sample for 16 hours at 4°C. A Cy5® conjugated goat anti-rat antibody was used as the secondary.

ab6160 at 1/1000 dilution staining Tubulin in human WBC cells by Immunocytochemistry/Immunofluorescence.

Cells were fixed in acetone and then blocked in 5% serum for 1 hour at 25°C. No permeabilization was done. The primary antibody was used at 1/1000 dilution in PBS-Tween and incubated with sample at 4°C for 16 hours. An Alexa Fluor® 594 conjugated goat polyclonal to rat IgG was used as secondary at 1/500 dilution.

This image was kindly supplied as part of the review submitted by Marko Kallio. ab6160 was used for immunofluorescence on male rat testis samples in order to visualize microtubules of meiotically dividing cells. The samples were fixed with 2% paraformaldehyde and 0.8% glutaraldehyde and the antibody was used at a dilution 1:2500 (red - tubulin, blue - DNA stained with DAPI).
ab6160 at a 1/200 staining Tubulin in mouse liver tissue sections by Immunohistochemistry (frozen sections) incubated for 9 hours at +4°C. Fixed in formaldehyde, permeabilized using 0.2% Triton X-100. Blocked using 2% BSA for 30 minutes at 20°C. Secondary used at a 1/200 dilution polyclonal Goat anti-rat IgG conjugated to Alexa Fluor® 555.

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