

## **Product datasheet**

# Anti-Tubulin antibody [YL1/2] - BSA and Azide free ab264519

### 4 Images

#### Overview

Product name	Anti-Tubulin antibody [YL1/2] - BSA and Azide free	
Description	Rat monoclonal [YL1/2] to Tubulin - BSA and Azide free	
Host species	Rat	
Tested applications	Suitable for: WB, IHC-P, ICC/IF, Flow Cyt (Intra)	
Species reactivity	Reacts with: Mouse, Rat, Human 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	
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	ab264519 is the carrier-free version of <u>ab6160</u> .	
	This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact <b>orders@abcam.com</b> .	
	Our <b><u>carrier-free</u></b> antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.	
	This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.	
	Use our <b>conjugation kits</b> for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.	
	This product is compatible with the Maxpar <sup>®</sup> Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar <sup>®</sup> is a trademark of Fluidigm Canada Inc.	
	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 +4°C. Do Not Freeze.
Storage buffer	Constituent: PBS
Carrier free	Yes
Purity	lgG fraction
Clonality	Monoclonal
Clone number	YL1/2
lsotype	lgG2a

#### Applications

The Abpromise guaranteeOur Abpromise guaranteecovers the use of ab264519 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		1/5000 - 1/10000.
IHC-P		Use at an assay dependent concentration.
ICC/IF		1/1000. (see PMID: 16230461)
Flow Cyt (Intra)		Use $1\mu g$ for $10^6$ cells. <u>ab18450</u> - Rat monoclonal lgG2a, 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

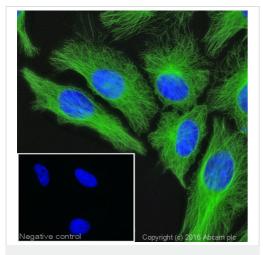
group. Also monoglycylated but not polyglycylated due to the absence of functional TTLL10 in human. Monoglycylation is mainly limited to tubulin incorporated into axonemes (cilia and flagella) whereas glutamylation is prevalent in neuronal cells, centrioles, axonemes, and the mitotic spindle. Both modifications 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 [YL1/2] - BSA and Azide free (ab264519)

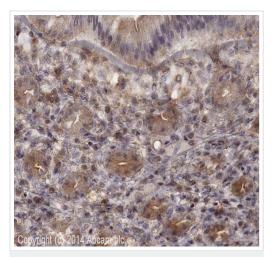
ICC/IF image of <u>ab6160</u> stained HeLa (Human epithelial cell line from cervix adenocarcinoma) cells.

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<sup>®</sup> 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.

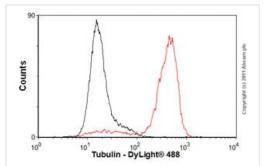
The negative control (inset) is a secondary-only assay to demonstrate low non-specific binding of the secondary antibody.

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, L-arginine and sodium azide (**ab6160**).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Tubulin antibody [YL1/2] -BSA and Azide free (ab264519)



Flow Cytometry (Intracellular) - Anti-Tubulin antibody [YL1/2] - BSA and Azide free (ab264519)

IHC image of Tubulin staining in human colon formalin fixed paraffin embedded tissue section\*, performed on a Leica Bond<sup>™</sup> 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  $\mu$ g/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 This data was developed using the same antibody clone in a different buffer formulation containing PBS, L-arginine and sodium azide (**ab6160**).

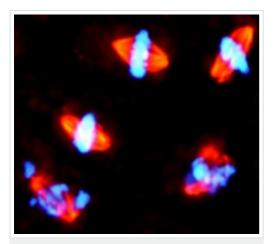
Overlay histogram showing HeLa (Human epithelial cell line from cervix adenocarcinoma) cells stained with **ab6160** (red line).

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  $\mu$ g/1x10<sup>6</sup> cells) for 30 minutes at 22°C. The secondary antibody used was DyLight<sup>®</sup> 488 goat anti-rat lgG (H+L) (**ab98386**) at 1/500 dilution for 30 minutes at 22°C. Isotype control antibody (black line) was rat lgG2a [aRTK2758] (**ab18450**, 1  $\mu$ g/1x10<sup>6</sup> cells) used under the same conditions.

Acquisition of >5,000 events was performed.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, L-arginine and sodium

#### azide (ab6160).



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 deviding 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).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, L-arginine and sodium azide (<u>ab6160</u>).

Immunocytochemistry/ Immunofluorescence - Anti-Tubulin antibody [YL1/2] - BSA and Azide free (ab264519)

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