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

15 Abreviews | 51 References | 8 Images | URL for this product: http://www.abcam.com/Tubulin-antibody-YL1-2-Loading-Control-ab6160.html

Product overview

Description	Rat monoclonal [YL1/2] to Tubulin - Loading Control
Host species	Rat
Specificity	This antibody detects the tyrosinated form of the alpha-tubulin subunit. It has equal affinity for fixed microtubules (formaldehyde or glutaraldehyde) and native microtubules. The antibody recognises an extremely short amino acid sequence Glu-Glu-Phe(OH) which can be inserted into the C-terminal domain of fusion proteins.
Tested applications	ELISA, IHC-Fr, IP, RIA, WB, Flow Cyt, ICC/IF, IHC (PFA fixed), IHC-P, IHC (Methanol fixed)
Cross reactivity	**Reacts with** Mouse, Human, Pig, Saccharomyces cerevisiae, Xenopus laevis, Caenorhabditis elegans, Fruit fly (Drosophila melanogaster), Schizosaccharomyces pombe **Predicted to work with** a wide range of other species, all Mammals
Immunogen	Full length native protein (purified) (S. cerevisiae).
Epitope	A linear sequence requiring an aromatic residue at the C terminus, with the two adjacent amino acids being negatively charged (represented by Gly-Gly-Tyr in Tyr-Tubulin).
Positive control	This antibody gave a positive signal in HeLa, NIH 3T3 and PC12 whole cell lysates
General notes	This antibody can be used both as a Loading Control and as a Microtubule Marker.

Properties

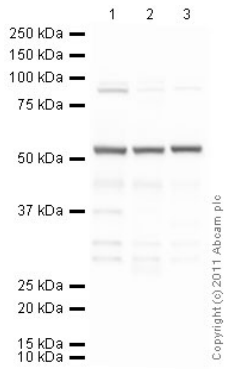
Form	Liquid
Storage instructions	Store at +4ºC short term (1-2 weeks). Aliquot and store at -20ºC or -80ºC. Avoid repeated freeze / thaw cycles.
Storage buffer	Preservative: 0.01% Sodium Azide Constituents: PBS, pH 7.4 See the website for more information on MSDS for this product.
Purity	IgG fraction
Primary antibody notes	This antibody can be used as a loading control on Western blots (Allen *et al.*) and is not detected 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 *E. coli* rec A and oxidized actin.
Clonality	Monoclonal
Clone number	YL1/2
Isotype	IgG2a

Applications

ELISA	ELISA: Use at an assay dependent dilution.
IHC-Fr	IHC-Fr: Use at an assay dependent dilution.
IP	IP: Use at an assay dependent dilution.
RIA	RIA: Use at an assay dependent dilution.
WB	WB: 1/5000 - 1/10000.
Flow Cyt	Flow Cyt: Use 1µg for 10^6 cells.
ICC/IF	ICC/IF: 1/1000((see PMID: 16230461))
IHC (PFA fixed)	IHC (PFA fixed): Use a concentration of 5 µg/ml((from PubMed:16966421))
IHC-P	IHC-P: Use at an assay dependent dilution.

IHC (Methanol fixed) IHC (Methanol fixed): 1/100((from PubMed:16943269).)

Images (See the website for higher resolution images of this product)



All lanes : Anti-Tubulin antibody [YL1/2] - 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 : PC12 (Rat adrenal pheochromocytoma cell line) Whole Cell Lysate

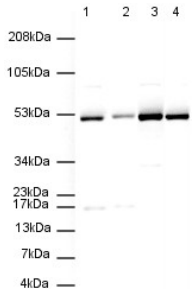
Lysates/proteins at 10 µg per lane.

Secondary
Peroxidase Conjugated AffiniPure 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



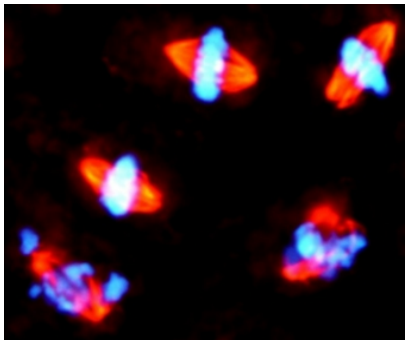
Lanes 1 & 3 : Anti-Tubulin antibody [YL1/2] - Loading Control (ab6160) at 1/5000 dilution
Lanes 2 & 4 : Anti-Tubulin antibody [YL1/2] - Loading Control (ab6160) at 1/10000 dilution

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 3 : BALB/3T3 (Mouse embryonic fibroblast cell line) Whole Cell Lysate (ab7901)
Lane 4 : BALB/3T3 (Mouse embryonic fibroblast cell line) Whole Cell Lysate (ab7901)

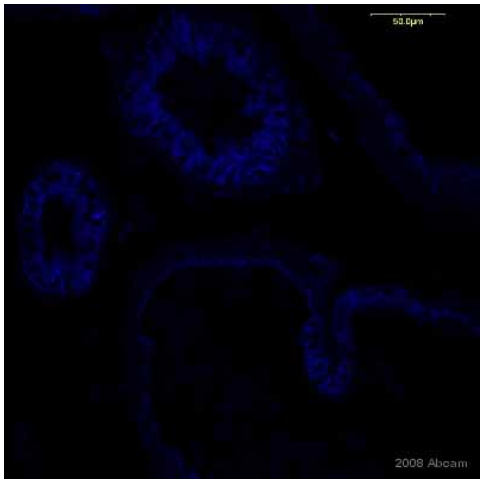
Lysates/proteins at 20 µg per lane.

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

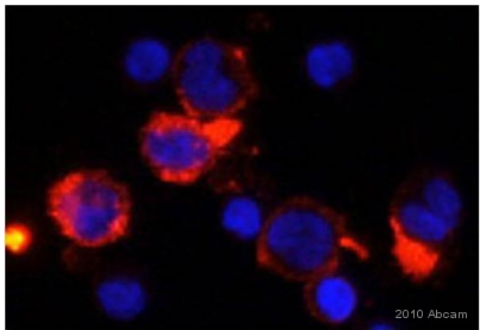


This image was kindly supplied as part of the review submitted by Marko Kallio. Ab6160 was used for immunofluorescence on male rat testis samples in order to visualize microtubules of meiotically deviding cells. The samples were fixed with 2% paraformaldehyde and 0.8% glutaraldehyde and the antibody was used at a dilution 1:2500 (red - tubulin, blue - DNA stained with DAPI).



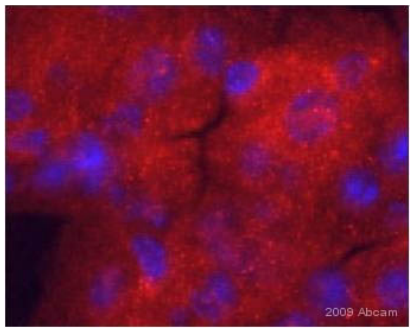
ab6160 staining mouse prostate tissue sections by IHC-P. The tissue was serial sectioned at 6 microns, formaldehyde fixed and subjected to heat mediated antigen retrieval prior to blocking in 3% peroxidase for 5 minutes at 27°C. The primary antibody was diluted 1/500 and incubated with the sample for 16 hours at 4°C. A Cy5® conjugated goat anti-rat antibody was used as the secondary.

This image is courtesy of an anonymous Abreview

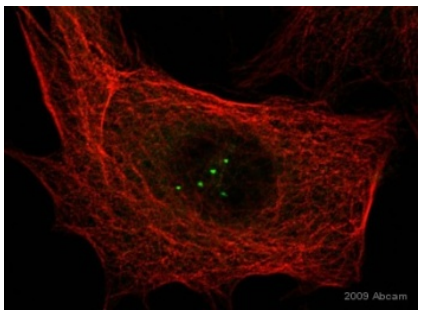


ab6160 at 1/1000 dilution staining Tubulin in human WBC cells by Immunocytochemistry/ Immunofluorescence. Cells were fixed in acetone and then blocked in 5% serum for 1 hour at 25°C. No permeabilization was done. The primary antibody was used at 1/1000 dilution in PBS-Tween and incubated with sample 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 is courtesy of an anonymous Abreview.

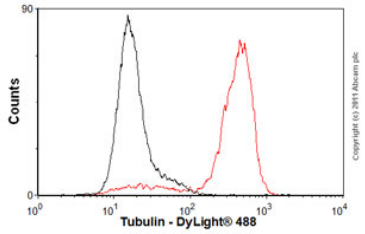


ab6160 at a 1/200 staining Tubulin in mouse liver tissue sections by Immunohistochemistry (frozen sections) incubated for 9 hours at +4°C. Fixed in formaldehyde, permeabilized using 0.2% Triton X-100. Blocked using 2% BSA for 30 minutes at 20°C. Secondary used at a 1/200 dilution polyclonal Goat anti-rat IgG conjugated to Alexa Fluor 555.
This image is courtesy of an anonymous abreview.



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 Cyt3®-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.

This Image is courtesy of an anonymous Abreview



Overlay histogram showing HeLa cells stained with ab6160 (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 (ab6160, 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.

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Quality is important to Abcam. If this product does not perform as described on this datasheet, notify us within 120 days of delivery using the online form, so that we can help you or offer you a replacement or a refund.