

## Product datasheet

# Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker ab52866

Recombinant RabMAb

★★★★★ [29 Abreviews](#) [169 References](#) [18 Images](#)

### Overview

<b>Product name</b>	Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker
<b>Description</b>	Rabbit monoclonal [EP1332Y] to alpha Tubulin - Microtubule Marker
<b>Host species</b>	Rabbit
<b>Specificity</b>	ab52866 is expected to recognise most alpha tubulin proteins and not only TUBA4A.
<b>Tested applications</b>	<b>Suitable for:</b> ICC/IF, Flow Cyt (Intra), WB, IHC-P
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Rat, Human, Pig, Drosophila melanogaster
<b>Immunogen</b>	Synthetic peptide within Human alpha Tubulin aa 1-100 (N terminal). The exact sequence is proprietary. Database link: <a href="#">P68366</a>
<b>Positive control</b>	WB- HeLa, HEK-293, HepG2, Caco2, NIH/3T3, PC-12, RAW 264.7, PC-12, C6 Jurkat and HEK-293T whole cell lysates; human fetal kidney lysate; Mouse and rat brain lysate; Pig skeletal muscle lysates; IHC-P: Pig kidney tissue; rat kidney tissue; mouse kidney tissue; human breast cancer and stomach tissue; IHC-Fr: Rat kidney tubule tissue; Flow Cyt (intra): HepG2 cells; ICC/IF: HUVEC, HeLa and 293 cells.
<b>General notes</b>	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"><li>- High batch-to-batch consistency and reproducibility</li><li>- Improved sensitivity and specificity</li><li>- Long-term security of supply</li><li>- Animal-free production</li></ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a>.</p> <p><b>We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.</b></p>

### Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	Shipped at 4°C. Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
<b>Storage buffer</b>	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.5% BSA
<b>Purity</b>	Protein A purified
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	EP1332Y
<b>Isotype</b>	IgG

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab52866 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
ICC/IF	★★★★★ (7)	1/250 - 1/500.
Flow Cyt (Intra)		1/20 - 1/50. <b>ab172730</b> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★★ (19)	1/1000 - 1/50000. Detects a band of approximately 50 kDa (predicted molecular weight: 50 kDa).
IHC-P	★★★★★ (1)	Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

## Target

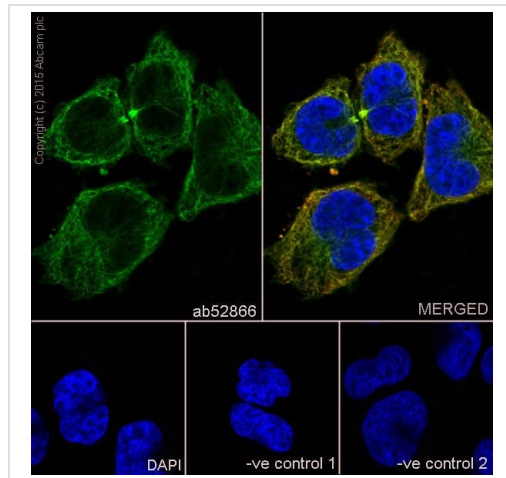
<b>Function</b>	Tubulin is the major constituent of microtubules. It binds two moles of GTP, one at an exchangeable site on the beta chain and one at a non-exchangeable site on the alpha chain.
<b>Sequence similarities</b>	Belongs to the tubulin family.
<b>Post-translational modifications</b>	Some glutamate 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 TLL10 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. Acetylation of alpha chains 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

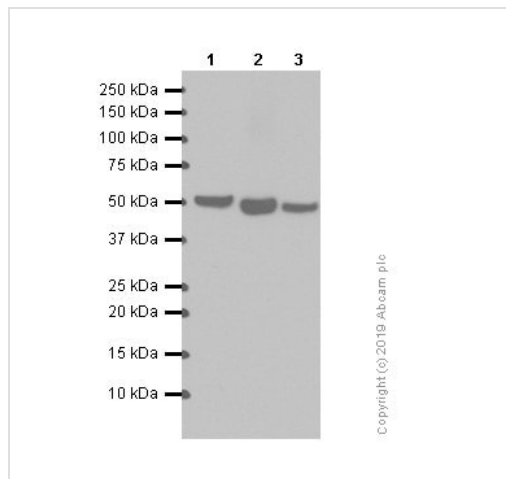


Immunocytochemistry/ Immunofluorescence - Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866)

Immunofluorescent analysis of 4% paraformaldehyde-fixed HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling alpha Tubulin with ab52866 at 1/500 dilution. The cells were permeabilised with 0.1% Triton X-100. Anti-rabbit Alexa Fluor® 488 ([ab150077](#)) at 1/400 dilution was used as the secondary antibody (green). The confocal image shows microtubules staining on HeLa cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with [ab7291](#) (anti-Tubulin mouse mAb) at 1/500 and anti-mouse AlexaFluor® 594 ([ab150120](#)) at 1/500 dilution (red).

The negative controls are as follows:

1. ab52866 at 1/500 dilution followed by anti-mouse AlexaFluor® 594 ([ab150120](#)) at 1/500 dilution.
2. [ab7291](#) (anti-Tubulin mouse mAb) at 1/500 dilution followed by anti-rabbit Alexa Fluor® 488 ([ab150077](#)) at 1/400 dilution.



Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866)

**All lanes :** Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866) at 1/10000 dilution

**Lane 1 :** HeLa (Human cervix adenocarcinoma epithelial cell) Whole cell lysates

**Lane 2 :** PC-12 (Rat adrenal gland heochromocytoma) whole cell lysates

**Lane 3 :** NIH/3T3( Mouse embryonic fibroblast)whole cell lysates

Lysates/proteins at 15 µg per lane.

### Secondary

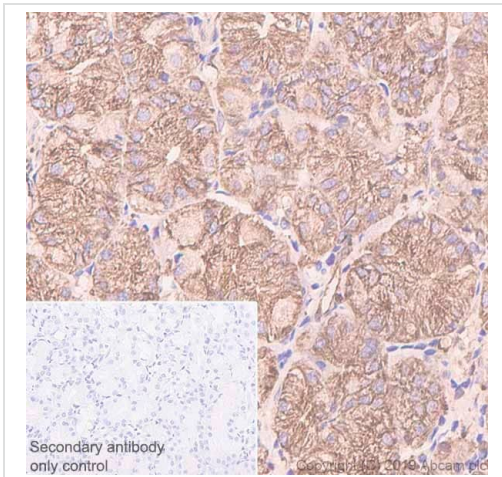
**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/20000 dilution

**Predicted band size:** 50 kDa

**Observed band size:** 52 kDa

**Exposure time:** 1 second

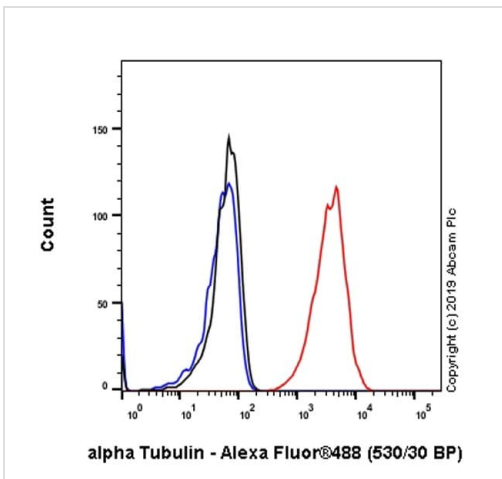
Blocking/Diluting buffer and concentration: 5% NFD/MTBST



Immunohistochemical analysis of paraffin-embedded Human stomach tissue labeling alpha Tubulin with ab52866 at followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Cytoplasmic staining on human stomach. The section was incubated with **ab229902** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND<sup>®</sup> RX instrument. Counterstained with Hematoxylin. Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).

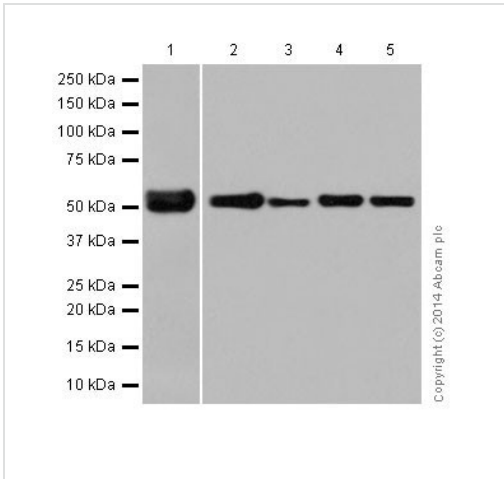
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866)



Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HepG2 (Human hepatocellular carcinoma epithelial cell) cells labelling alpha Tubulin with ab52866 at 1/2000 dilution (Red) compared with a Rabbit monoclonal IgG (**ab172730**) isotype control (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor<sup>®</sup> 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.

Flow Cytometry (Intracellular) - Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866)



Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866)

**All lanes :** Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866) at 1/5000 dilution

**Lane 1 :** Mouse brain lysates

**Lane 2 :** C6 (Rat glial tumor cell line) whole cell lysates

**Lane 3 :** RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysates

**Lane 4 :** PC-12 (Rat adrenal gland pheochromocytoma cell line) whole cell lysates

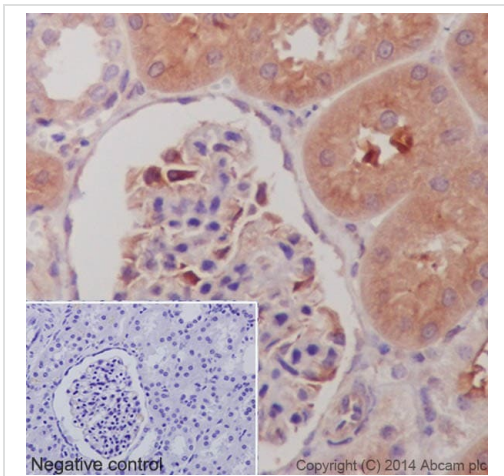
**Lane 5 :** NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysates

Lysates/proteins at 10 µg per lane.

### Secondary

**All lanes :** Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

**Predicted band size:** 50 kDa

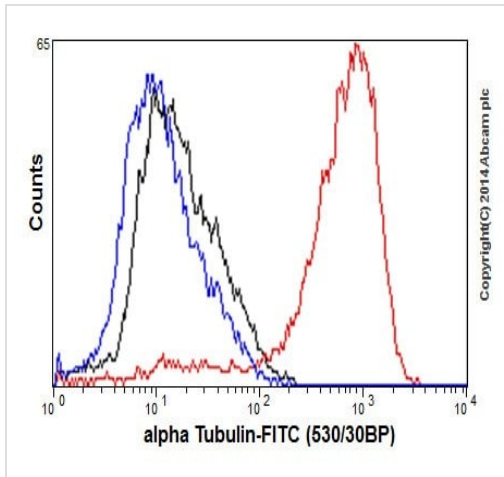


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866)

Immunohistochemistry analysis of paraffin-embedded Pig kidney tissue labeling alpha Tubulin with ab52866 at a 1/1000 dilution. Cytoplasmic staining on Pig kidney tubule and weak on glomerulus shown. **Anti-Rabbit HRP (ab97051)** used at a 1/100 dilution. Counter stained with Hematoxylin.

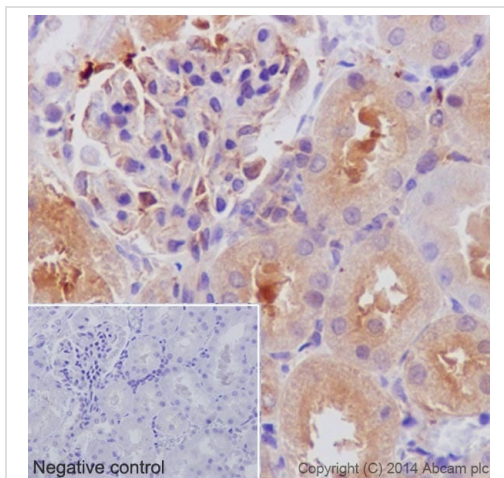
Inset image: negative control obtained using PBS instead of ab52866, secondary antibody is **Anti-Rabbit HRP (ab97051)** at 1/100 dilution.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866)

Intracellular Flow Cytometry analysis of 2% paraformaldehyde fixed HepG2 (human liver hepatocellular carcinoma cell line) cells labeling alpha Tubulin with ab52866 at 1/130 dilution (red line). Secondary antibody used is a goat anti rabbit IgG (FITC) at 1/150 dilution. The isotype control is rabbit monoclonal IgG (black line). The unlabeled control is cells without incubation with primary and secondary antibodies (blue line).



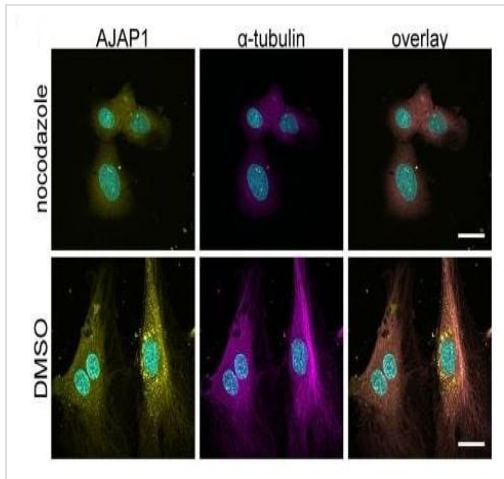
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866)

Immunohistochemistry analysis of paraffin-embedded Rat kidney tissue labeling alpha Tubulin with ab52866 at a 1/1000 dilution. Cytoplasmic staining on Rat kidney tubule and weak on glomerulus shown. Secondary antibody **Anti-Rabbit HRP (ab97051)** used at a 1/500 dilution. Counter stained with Hematoxylin.

Inset image: negative control obtained using PBS instead of ab52866, secondary antibody is **Anti-Rabbit HRP (ab97051)** at 1/500 dilution.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.





Immunocytochemistry/ Immunofluorescence - Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866)

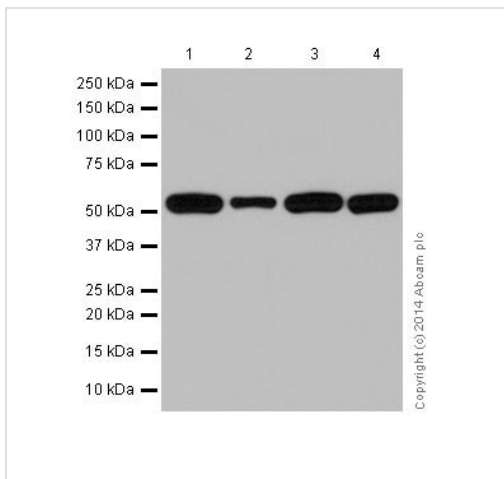
Hotte, K. et al Biol Open. 2017 Jun 15;6(6):723-731. doi: 10.1242/bio.022335 Reproduced under the Creative Commons license <http://creativecommons.org/licenses/by/3.0>

### AJAP1 co-localizes with microtubules in HUVECs

The association of AJAP1 with microtubules in HUVECs is lost upon microtubule destruction. Treatment with 12.5 μM nocodazole for 24 h shows destruction of the microtubule network and loss of AJAP1 tubular localization. For a negative control, HUVECs are treated with DMSO for 24 h. Cell nuclei were counterstained with DAPI (cyan). Microscope: Zeiss LSM 780; objective lens: 63×/1.40 oil; scale bar: 25 μm.

Incubated overnight at 4°C with ab52866.

(From Figure 3E of Hotte et al)



Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866)

**All lanes :** Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866) at 1/20000 dilution

**Lane 1 :** HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate

**Lane 2 :** HepG2 (human liver hepatocellular carcinoma cell line) whole cell lysate

**Lane 3 :** Jurkat (human T cell leukemia cell line from peripheral blood) whole cell lysate

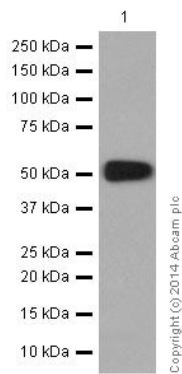
**Lane 4 :** HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate

Lysates/proteins at 20 μg per lane.

### Secondary

**All lanes :** Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

**Predicted band size:** 50 kDa



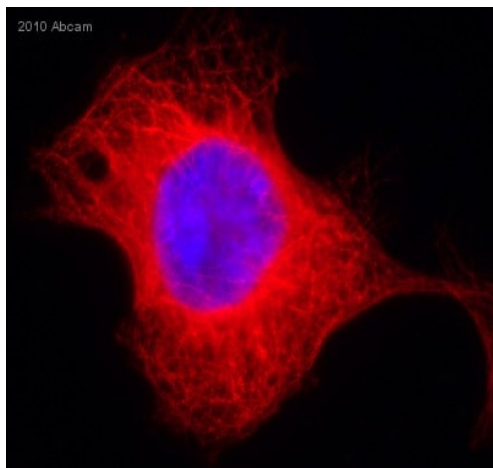
Western blot - Anti-alpha Tubulin antibody  
[EP1332Y] - Microtubule Marker (ab52866)

Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker  
(ab52866) at 1/50000 dilution + Rat brain lysates at 10 µg

### Secondary

Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at  
1/1000 dilution

**Predicted band size: 50 kDa**

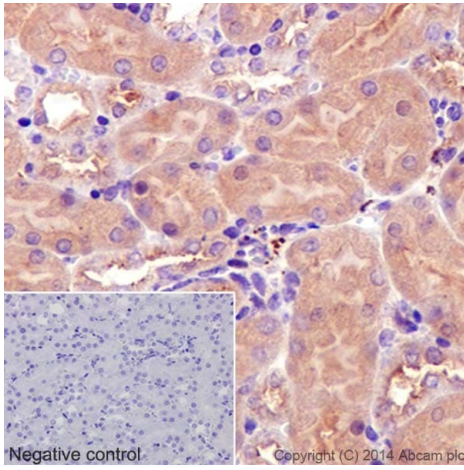


Immunocytochemistry/ Immunofluorescence - Anti-  
alpha Tubulin antibody [EP1332Y] - Microtubule  
Marker (ab52866)

This image is courtesy of an anonymous Abreview

ab52866 staining alpha Tubulin in 293 Human embryonic kidney cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde and blocked with 10% serum for 2 hours at 23°C. Samples were incubated with primary antibody (1/200 in 0.5% saponin) for 2 hours at 23°C. An Alexa Fluor<sup>®</sup>555-conjugated Goat anti-rabbit IgG polyclonal (1/500) was used as the secondary antibody. Nuclei were counterstained with DAPI.



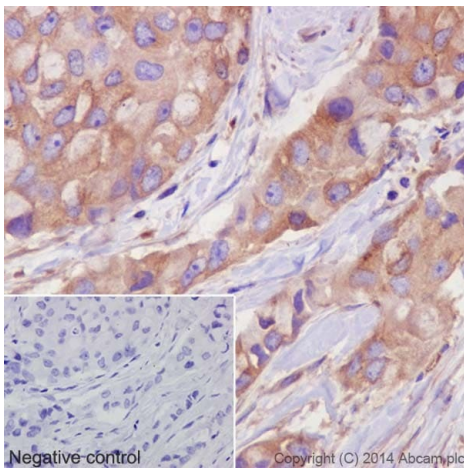


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866)

Immunohistochemistry analysis of paraffin-embedded Mouse kidney tissue labeling alpha Tubulin with ab52866 at a 1/1000 dilution. Cytoplasmic staining on Mouse kidney tubule shown. Secondary antibody **Anti-Rabbit HRP (ab97051)** used at a 1/500 dilution. Counter stained with Hematoxylin.

Inset image: negative control obtained using PBS instead of ab52866, secondary antibody is **Anti-Rabbit HRP (ab97051)** at 1/500 dilution.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

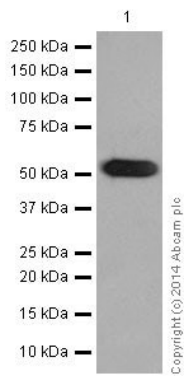


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866)

Immunohistochemistry analysis of paraffin-embedded Human breast cancer labeling alpha Tubulin with ab52866 at a 1/1000 dilution. Cytoplasmic staining on cancer cells shown. Secondary antibody **ab97051** Goat Anti-Rabbit IgG H&L (HRP) used at a 1/500 dilution. Counter stained with Hematoxylin.

Inset image: negative control obtained using PBS instead of ab52866, secondary antibody is Anti-Rabbit HRP (**ab97051**) at 1/500 dilution.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



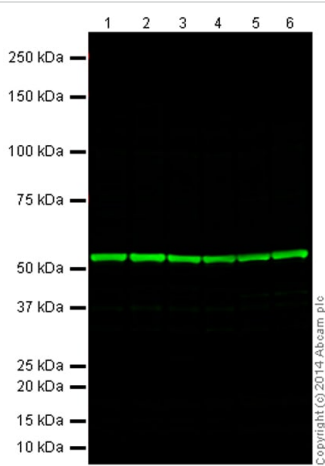
Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866)

Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866) at 1/50000 dilution + Human fetal kidney lysates at 10 µg

**Secondary**

Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

**Predicted band size:** 50 kDa



Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866)

**All lanes :** Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866) at 1/1000 dilution

**Lane 1 :** HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate

**Lane 2 :** HEK293 (Human embryonic kidney cell line) Whole Cell Lysate

**Lane 3 :** HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate

**Lane 4 :** Caco 2 (Human colonic carcinoma cell line) Whole Cell Lysate

**Lane 5 :** NIH/3T3 (Mouse embryonic fibroblast cell line) Whole Cell Lysate

**Lane 6 :** PC-12 (Rat adrenal pheochromocytoma cell line) Whole Cell Lysate

Lysates/proteins at 20 µg per lane.

**Secondary**

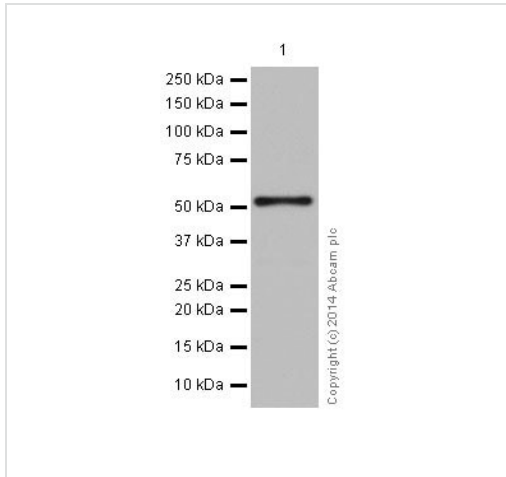
**All lanes :** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 790) (**ab175781**) at 1/10000 dilution

**Predicted band size:** 50 kDa

**Observed band size:** 52 kDa

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes.

The membrane was then blocked for an hour using Licor blocking buffer before being incubated with ab52866 overnight at 4°C. Antibody binding was detected using **Anti-Rabbit Alexa Fluor® 790 (ab175781)** at a 1:10,000 dilution for 1hr at room temperature and then imaged using the Licor Odyssey CLx.



Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866)





Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866) at 1/5000 dilution + Pig skeletal muscle lysates at 20 µg

### Secondary

Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

**Predicted band size: 50 kDa**

Why choose a recombinant antibody?

 <p><b>Research with confidence</b> Consistent and reproducible results</p>	 <p><b>Long-term and scalable supply</b> Recombinant technology</p>
 <p><b>Success from the first experiment</b> Confirmed specificity</p>	 <p><b>Ethical standards compliant</b> Animal-free production</p>

Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866)

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