abcam

Product datasheet

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

Recombinant RabMAb

24 Abreviews 70 References 16 Images

Overview

Product name	Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker
Description	Rabbit monoclonal [EP1332Y] to alpha Tubulin - Microtubule Marker
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, IHC - Wholemount, WB, IP, Flow Cyt, IHC-P, IHC-Fr
Species reactivity	**Reacts with:** Mouse, Rat, Human, Pig, Drosophila melanogaster
Immunogen	Synthetic peptide within Human alpha Tubulin aa 1-100 (N terminal). The exact sequence is proprietary.
Positive control	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 tissue. IHC-Fr: Rat kidney tubule tissue. Flow cyt: HepG2 cells. IP: HeLa whole cell extract. ICC/IF: HUVEC, HeLa and 293 cells.
General notes	A trial size is available to purchase for this antibody. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents. **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.** This product is a recombinant rabbit monoclonal antibody.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide

	Constituents: 59% PBS, 40% Glycerol, 0.5% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP1332Y
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab52866** in the following tested applications.

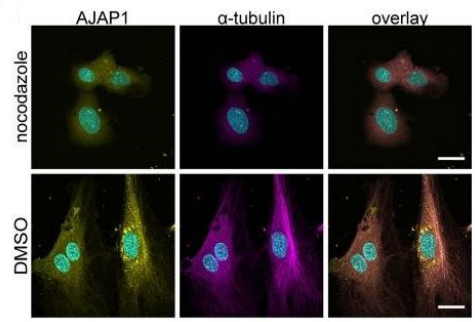
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF	★★★★☆	1/250 - 1/500.
IHC - Wholemount	★★★★★	Use at an assay dependent concentration.
WB	★★★★★	1/1000 - 1/50000. Detects a band of approximately 50 kDa (predicted molecular weight: 50 kDa).
IP		1/50.
Flow Cyt		1/20 - 1/50. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-P	★★★★☆	Use at an assay dependent concentration.
IHC-Fr	★★★★★	Use at an assay dependent concentration. PubMed: 21933451

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	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 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)

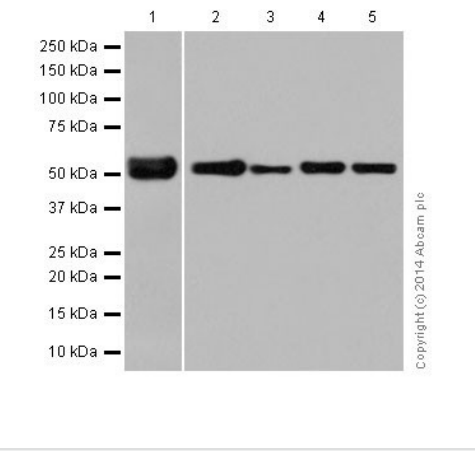
Hotte, K. et al Biol Open. 2017 Jun 15;6(6):723-731. doi: 10.1242/bio.022335

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)

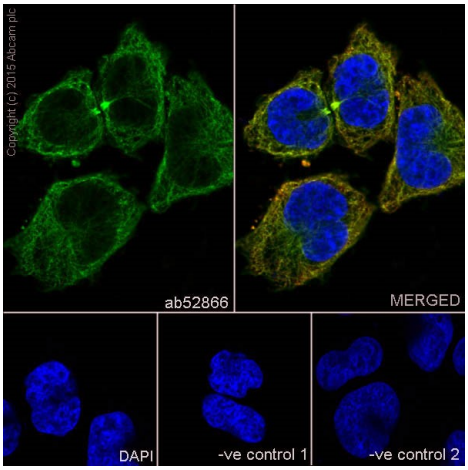
All lanes : Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866) at 1/5000 dilution

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.

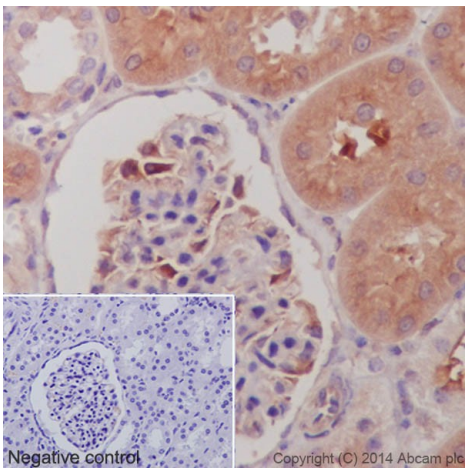
Secondary
All lanes : Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1000 dilution

Predicted band size: 50 kDa



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

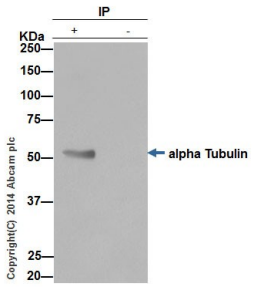
Immunofluorescent analysis of 4% paraformaldehyde-fixed HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling alpha Tubulin with ab52866 at 1/500 dilution. The cells were permeabilised with 0.1% Triton X-100. Anti-rabbit Alexa Fluor® 488 (ab150077) at 1/400 dilution was used as 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.



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.

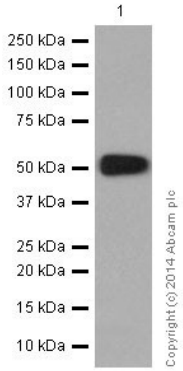
Inset image: negative control obtained using PBS instead of ab52866, secondary antibody is Anti-Rabbit HRP (ab97051) at 1/100 dilution.



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

alpha Tubulin was immunoprecipitated from 1mg of HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell extract using ab52866 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab52866 at 1/1000 dilution. Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG, was used as secondary antibody at 1/1500 dilution.
Lane 1 Hela whole cell extract, Lane 2 PBS instead of whole cell extract.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.



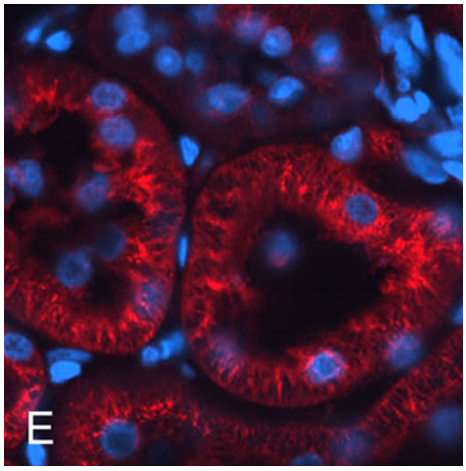
Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866)

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

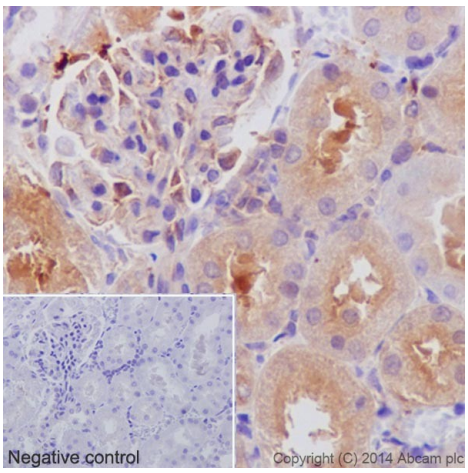
Predicted band size: 50 kDa



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

Image from Akimoto Y et al., Clin Proteomics. 2011 Sep 21;8(1):15. Fig 3.; doi:10.1186/1559-0275-8-15; 21 September 2011, Clinical Proteomics 2011, 8:15

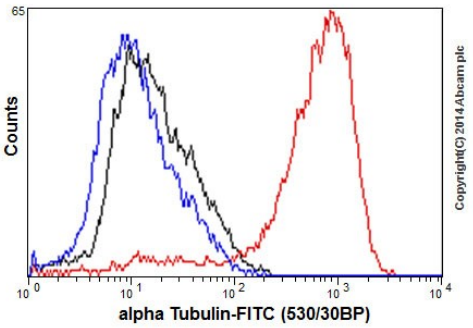
Immunohistochemical analysis of rat kidney tubule tissue, staining alpha Tubulin with ab52866.



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

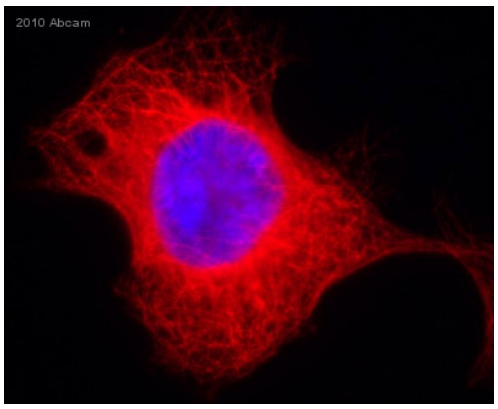
Immunohistochemistry analysis of paraffin-embedded Rat kidney tissue labeling alpha Tubulin with ab52866 at a 1/1000 dilution. Cytoplasmic staining on Rat kidney tubule and weak on glomerulus shown. Secondary antibody Anti-Rabbit HRP (ab97051) used at a 1/500 dilution. Counter stained with Hematoxylin.

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



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

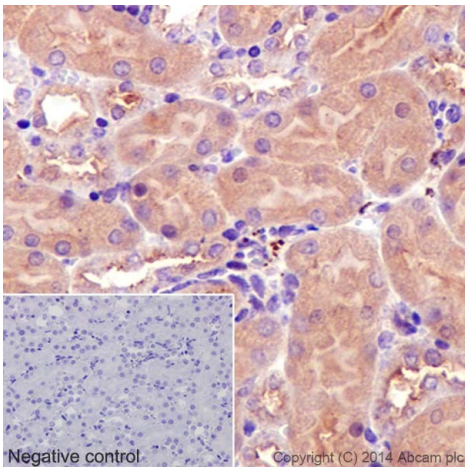
Flow cytometry analysis of 2% paraformaldehyde fixed HepG2 (human liver hepatocellular carcinoma cell line) cells labeling alpha Tubulin with ab52866 at 1/130 dilution (red line). Secondary antibody used is a goat anti rabbit IgG (FITC) at 1/150 dilution. The isotype control is rabbit monoclonal IgG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



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

This image is courtesy of an anonymous Abreview

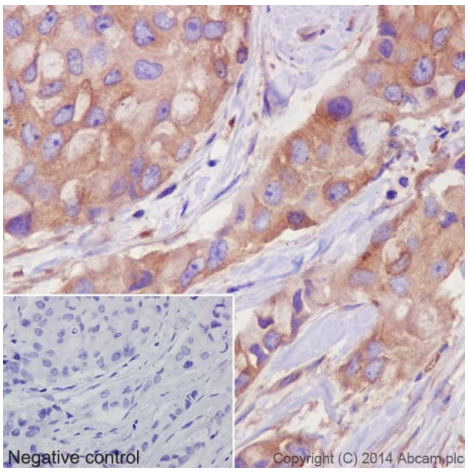
ab52866 staining alpha Tubulin in 293 Human embryonic kidney cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde and blocked with 10% serum for 2 hours at 23°C. Samples were incubated with primary antibody (1/200 in 0.5% saponin) for 2 hours at 23°C. An Alexa Fluor®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.

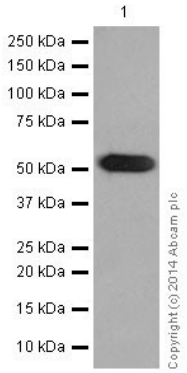
Inset image: negative control obtained using PBS instead of ab52866, secondary antibody is Anti-Rabbit HRP (ab97051) at 1/500 dilution.



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.



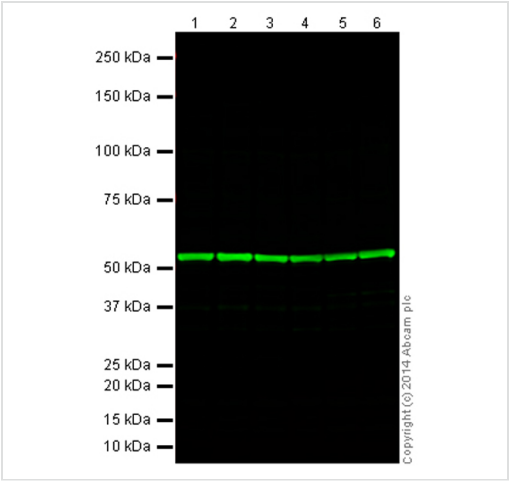
Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866)

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

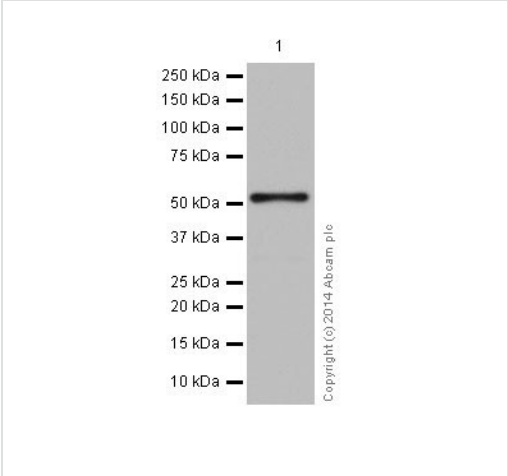
Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2 : HEK293 (Human embryonic kidney cell line) Whole Cell Lysate
Lane 3 : HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate
Lane 4 : Caco 2 (Human colonic carcinoma cell line) Whole Cell Lysate
Lane 5 : 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
why is the actual band size different from the predicted?

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.



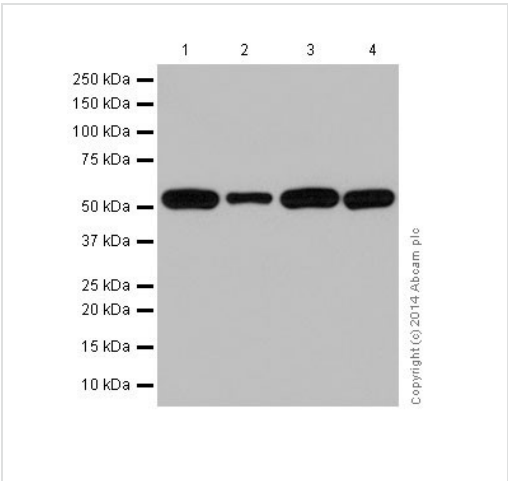
Western blot - Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866)

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



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

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