abcam

Product datasheet

Anti-alpha Tubulin (acetyl K40) antibody [EPR16772] ab179484

Recombinant RabMAb

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Overview

Product name	Anti-alpha Tubulin (acetyl K40) antibody [EPR16772]
Description	Rabbit monoclonal [EPR16772] to alpha Tubulin (acetyl K40)
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC/IF, IP, IHC-P, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, C6 and NIH/3T3 whole cell lysates (treated with 500 ng/ml Trichostatin A for 4 hours); Mouse brain, kidney and spleen lysates; Rat brain and heart lysates; Human fetal heart and fetal kidney lysates. IHC-P: Human and Mouse cerebral cortex tissue; rat cerebellum tissue. IF: HeLa cells treated with 50 ug/ml Trichostatin A for 4 hours. Flow: HeLa cells treated with 500ng/ml Trichostatin A for 4 hours. IP: HeLa treated with 500 ng/ml Trichostatin A for 4 hours.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

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 long term. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.01% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), 0.05% BSA, 59% PBS
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR16772
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab179484 in the following tested applications.

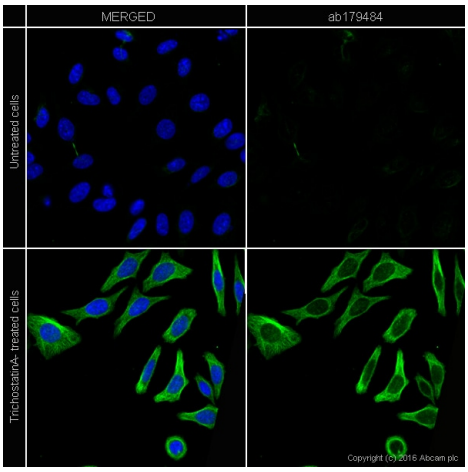
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/2000. Detects a band of approximately 52 kDa (predicted molecular weight: 50 kDa).
ICC/IF	★★★★☆ (1)	1/500.
IP		1/70.
IHC-P	★★★★★ (3)	1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Flow Cyt (Intra)		1/240. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Target

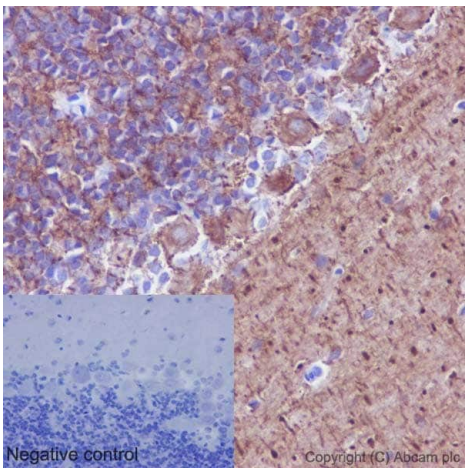
Function	Tubulin is the major constituent of microtubules. It binds two moles of GTP, one at an exchangeable site on the beta chain and one at a non-exchangeable site on the alpha chain.
Sequence similarities	Belongs to the tubulin family.
Post-translational modifications	Some glutamate residues at the 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 (acetyl K40) antibody [EPR16772] (ab179484)

ab179484 stained in Hela cells. Untreated and Trichostatin A treated (50ug/ml, 4 hours) cells were fixed with 4% paraformaldehyde (10min) at room temperature and incubated with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% triton for 1h at room temperature to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab179484 at 1/500 dilution overnight at +4°C. The secondary antibody was **ab150177** used at 1 ug/ml for 1hour at room temperature (colored green). DAPI was used to stain the cell nuclei (colored blue) at a concentration of 1.43µM for 1hour at room temperature.

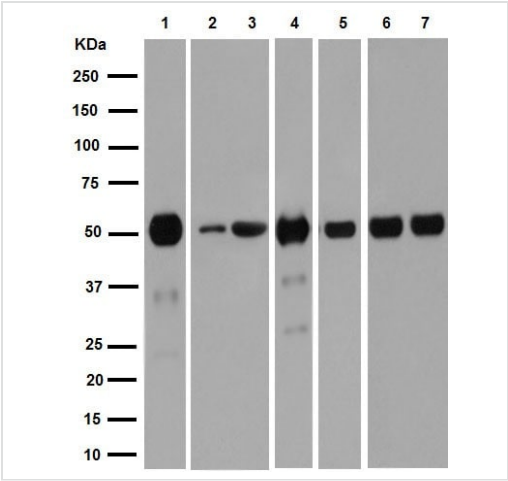


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha Tubulin (acetyl K40) antibody [EPR16772] (ab179484)

Immunohistochemical analysis of paraffin-embedded Rat cerebellum tissue labeling alpha Tubulin (acetyl K40) with ab179484 at 1/1000 dilution, followed by prediluted HRP Polymer for Rabbit/Mouse IgG. Cytoplasmic staining is observed on Purkinje cells of cerebellum. Counter stained with Hematoxylin.

Negative control: Using PBS instead of primary ab, secondary ab is prediluted HRP Polymer for Rabbit/Mouse IgG.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-alpha Tubulin (acetyl K40) antibody [EPR16772] (ab179484)

All lanes : Anti-alpha Tubulin (acetyl K40) antibody [EPR16772] (ab179484) at 1/2000 dilution

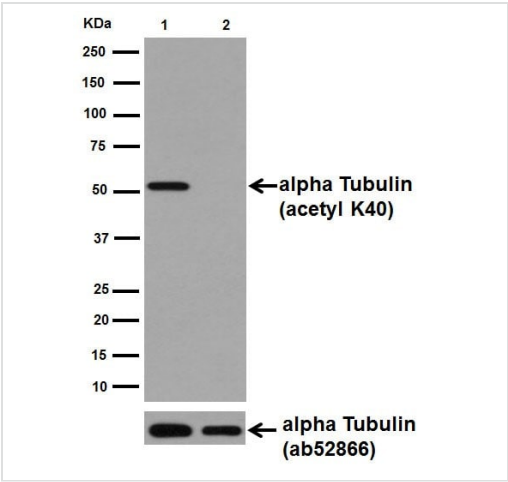
Lane 1 : Mouse brain lysate
Lane 2 : Mouse kidney lysate
Lane 3 : Mouse spleen lysate
Lane 4 : Rat brain lysate
Lane 5 : Rat heart lysate
Lane 6 : Human fetal heart lysate
Lane 7 : Human fetal kidney lysate

Lysates/proteins at 10 µg per lane.

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

Predicted band size: 50 kDa
Observed band size: 52 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-alpha Tubulin (acetyl K40) antibody [EPR16772] (ab179484)

All lanes : Anti-alpha Tubulin (acetyl K40) antibody [EPR16772] (ab179484) at 1/20000 dilution

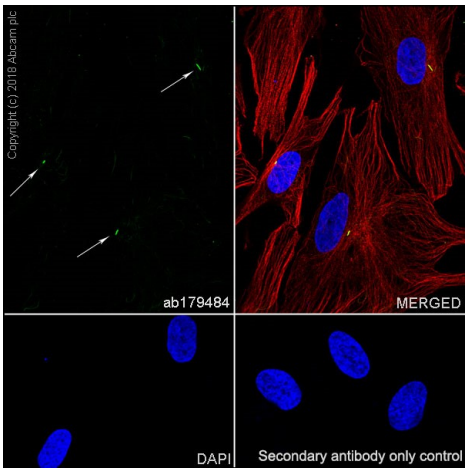
Lane 1 : HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate treated with 500 ng/ml Trichostatin A for 4 hours
Lane 2 : Untreated HeLa whole cell lysate

Lysates/proteins at 10 µg per lane.

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

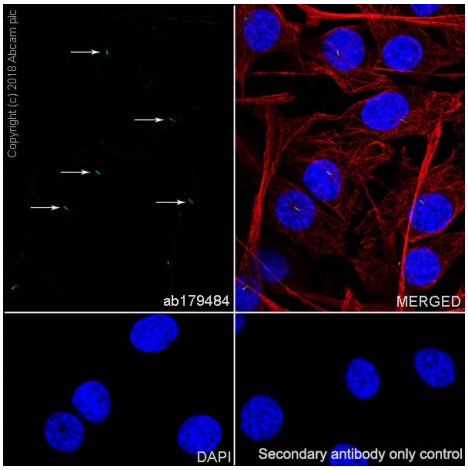
Predicted band size: 50 kDa
Observed band size: 52 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.



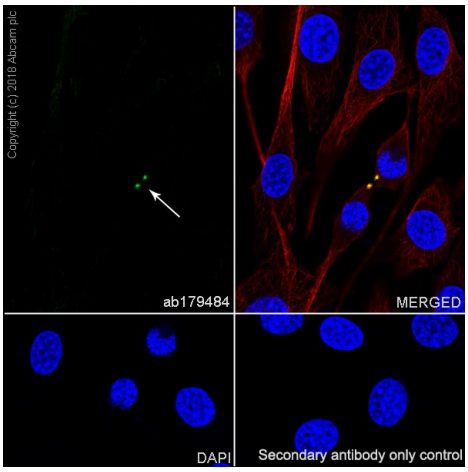
Immunocytochemistry/ Immunofluorescence - Anti-alpha Tubulin (acetyl K40) antibody [EPR16772] (ab179484)

Ab179484 staining alpha Tubulin in HFF-1 (Human skin fibroblast) cell line by ICC/IF (Immunocytochemistry/Immunofluorescence). The cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% TritonX-100. Samples were incubated with primary antibody at 1:20000 dilution. An AlexaFluor®488 Goat anti-Rabbit (**ab150077**) was used as a secondary antibody at 1:1000 dilution. An Anti-Alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594), **ab195889** was used as a counterstain at 1:200 dilution. DAPI was used as a nuclear counterstain. Confocal image showing cilia (arrows) staining in HFF-1 cells treated with starvation for 48 hours.



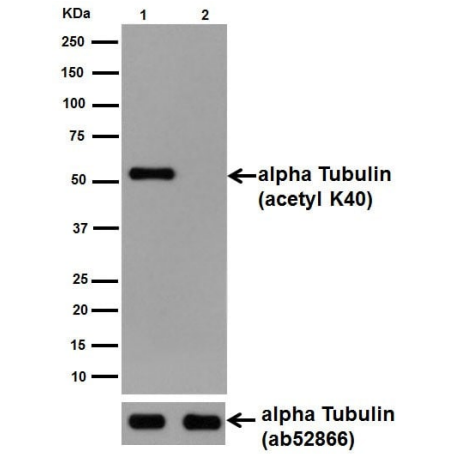
Immunocytochemistry/ Immunofluorescence - Anti-alpha Tubulin (acetyl K40) antibody [EPR16772] (ab179484)

Ab179484 staining alpha Tubulin in NIH/3T3 (mouse embryonic fibroblast) cell line by ICC/IF (Immunocytochemistry/Immunofluorescence).The cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% TritonX-100. Samples were incubated with primary antibody at 1:20000 dilution. An AlexaFluor®488 Goat anti-Rabbit (**ab150077**) was used as a secondary antibody at 1:1000 dilution. An Anti-Alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594), **ab195889** was used as a counterstain at 1:200 dilution. DAPI was used as a nuclear counterstain. Confocal image showing cilia (arrows) staining in NIH/3T3 cells treated with starvation for 48 hours.



Immunocytochemistry/ Immunofluorescence - Anti-alpha Tubulin (acetyl K40) antibody [EPR16772] (ab179484)

Ab179484 staining alpha Tubulin in NIH/3T3 (mouse embryonic fibroblast) cell line by ICC/IF (Immunocytochemistry/Immunofluorescence). The cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% TritonX-100. Samples were incubated with primary antibody at 1:20000 dilution. An AlexaFluor®488 Goat anti-Rabbit (**ab150077**) was used as a secondary antibody at 1:1000 dilution. An Anti-Alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594), **ab195889** was used as a counterstain at 1:200 dilution. DAPI was used as a nuclear counterstain. Confocal image showing midbody (arrows) staining in NIH/3T3 cells treated with starvation for 48 hours.



Western blot - Anti-alpha Tubulin (acetyl K40) antibody [EPR16772] (ab179484)

All lanes : Anti-alpha Tubulin (acetyl K40) antibody [EPR16772] (ab179484) at 1/20000 dilution

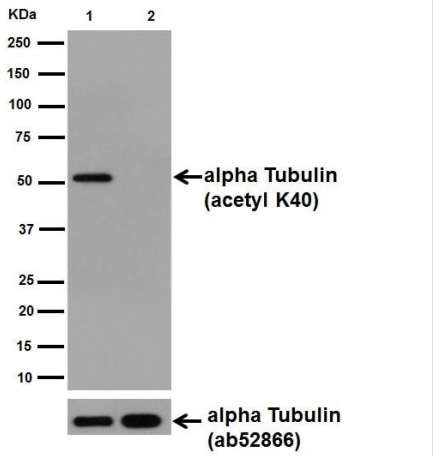
Lane 1 : C6 (Rat glial tumor cells) whole cell lysate treated with 500 ng/ml Trichostatin A for 4 hours
Lane 2 : Untreated C6 whole cell lysate

Lysates/proteins at 10 µg per lane.

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

Predicted band size: 50 kDa
Observed band size: 52 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-alpha Tubulin (acetyl K40) antibody [EPR16772] (ab179484)

All lanes : Anti-alpha Tubulin (acetyl K40) antibody [EPR16772] (ab179484) at 1/20000 dilution

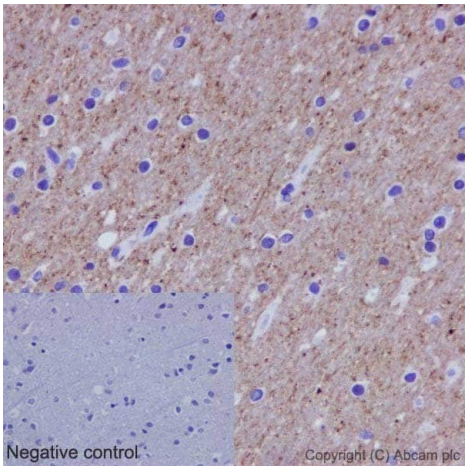
Lane 1 : NIH/3T3 (Mouse embyro fibroblast cells) whole cell lysate treated with 500 ng/ml Trichostatin A for 4 hours
Lane 2 : Untreated NIH/3T3 whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
Lane 1 : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution
Lane 2 : Goat Anti-Rabbit IgG, (H+L),Peroxidase conjugated at 1/1000 dilution

Predicted band size: 50 kDa
Additional bands at: 52 kDa. We are unsure as to the identity of these extra bands.

Blocking/dilution buffer: 5% NFDM/TBST.

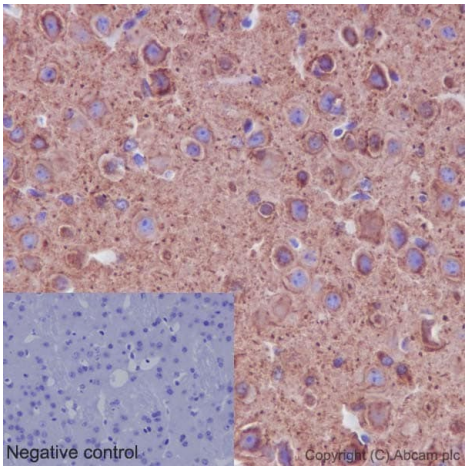


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha Tubulin (acetyl K40) antibody [EPR16772] (ab179484)

Immunohistochemical analysis of paraffin-embedded Human cerebral cortex tissue labeling alpha Tubulin (acetyl K40) with ab179484 at 1/1000 dilution, followed by prediluted HRP Polymer for Rabbit/Mouse IgG. Cytoplasmic staining is observed on neuron cells of Human brain tissue. Counter stained with Hematoxylin.

Negative control: Using PBS instead of primary ab, secondary ab is prediluted HRP Polymer for Rabbit/Mouse IgG.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

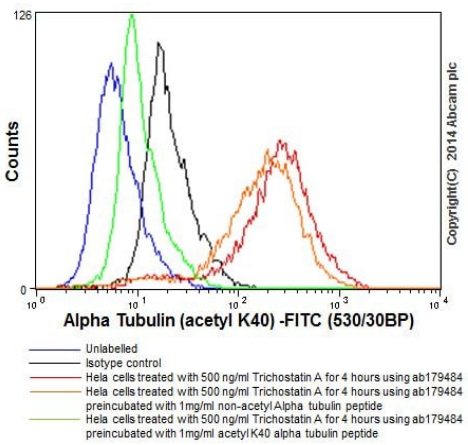


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha Tubulin (acetyl K40) antibody [EPR16772] (ab179484)

Immunohistochemical analysis of paraffin-embedded Mouse cerebral cortex tissue labeling alpha Tubulin (acetyl K40) with ab179484 at 1/1000 dilution, followed by prediluted HRP Polymer for Rabbit/Mouse IgG. Cytoplasmic staining is observed on neuron cells of Mouse cerebral cortex tissue. Counter stained with Hematoxylin.

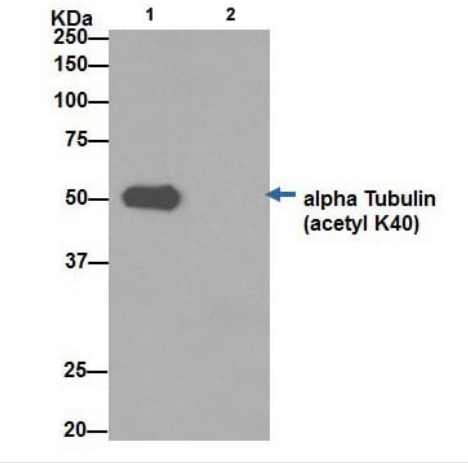
Negative control: Using PBS instead of primary ab, secondary ab is prediluted HRP Polymer for Rabbit/Mouse IgG.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-alpha Tubulin (acetyl K40) antibody [EPR16772] (ab179484)

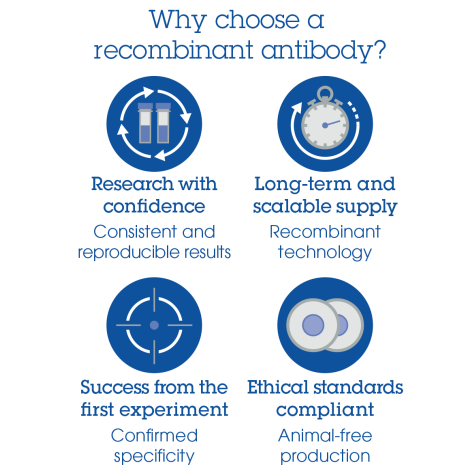
Intracellular flow cytometric analysis of 2% paraformaldehyde-fixed HeLa (Human epithelial cells from cervix adenocarcinoma) cells treated with 500 ng/ml Trichostatin A for 4 hourslabeling alpha Tubulin (acetyl K40)with ab179484 at 1/240 dilution (red line). Goat anti rabbit IgG (FITC) at 1/150 dilution was used as the secondary antibody. ab179484 preincubated with 1mg/ml acetyl Alpha tubulin (acetyl K40) peptide (green) or non-acetyl Alpha tubulin (acetyl K40) peptide (orange). The isotype control wasRabbit monoclonal IgG (black) and the unlabelled contol was cells without incubation with primary antibody and secondary antibody (blue).



Immunoprecipitation - Anti-alpha Tubulin (acetyl K40) antibody [EPR16772] (ab179484)

Alpha Tubulin was immunoprecipitated from 1mg of HeLa cells (Human epithelial cells from cervix adenocarcinoma) treated with 500 ng/ml Trichostatin A for 4 hours with ab179484 at 1/70 dilution. Western blot was performed from 10 µg of the immunoprecipitate using ab179484 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. Left lane: Hela whole cell extract. Right lane: PBS instead of Hela whole cell extract.

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



Anti-alpha Tubulin (acetyl K40) antibody [EPR16772] (ab179484)

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