abcam

Product datasheet

Anti-alpha Tubulin (acetyl K40) antibody [EPR16772] -BSA and Azide free ab209348

Recombinant RabMAb

10 Images

Overview

Product name Anti-alpha Tubulin (acetyl K40) antibody [EPR16772] - BSA and Azide free

Description Rabbit monoclonal [EPR16772] to alpha Tubulin (acetyl K40) - BSA and Azide free

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), WB, ICC/IF, IP, IHC-P

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 ab209348 is the carrier-free version of ab179484.

> Our carrier-free 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 cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our **conjugation kits** 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® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

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. Do Not Freeze.

Storage buffer pH: 7.2

Constituent: PBS

Carrier free Yes

Purity Protein A purified

ClonalityMonoclonalClone numberEPR16772

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab209348 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
Flow Cyt (Intra)		Use at an assay dependent concentration. ab199376 -Rabbit monoclonal lgG (Low endotoxin, Azide free), is suitable for use as an isotype control with this antibody.
WB		Use at an assay dependent concentration. Detects a band of approximately 52 kDa (predicted molecular weight: 50 kDa).
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

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)

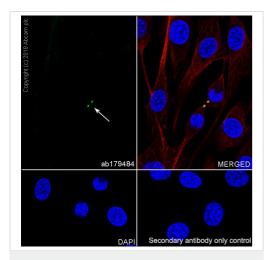
whereas glutamylation is prevalent in neuronal cells, centrioles, axonemes, and the mitotic spindle. Both modifications can coexist on the same protein on adjacent residues, and lowering glycylation levels increases polyglutamylation, and reciprocally. The precise function of such modifications is still 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 - Antialpha Tubulin (acetyl K40) antibody [EPR16772] -BSA and Azide free (ab209348)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab179484).

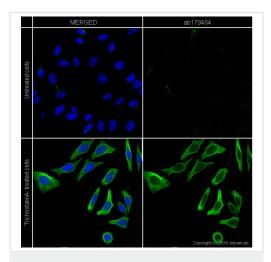
ab179484 MERGED

DAPI Secondary antibody only control

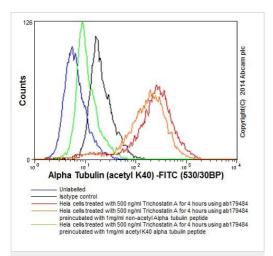
Immunocytochemistry/ Immunofluorescence - Antialpha Tubulin (acetyl K40) antibody [EPR16772] -BSA and Azide free (ab209348)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab179484).



Immunocytochemistry/ Immunofluorescence - Antialpha Tubulin (acetyl K40) antibody [EPR16772] -BSA and Azide free (ab209348)



Flow Cytometry (Intracellular) - Anti-alpha Tubulin (acetyl K40) antibody [EPR16772] - BSA and Azide free (ab209348)

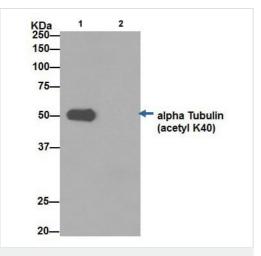
<u>ab179484</u> stained in Hela cells. Untreated and Trichostatin A treated (50ug/ml, 4 hours) cells were fixed with

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 <u>ab179484</u> at 1/500 dilution overnight at +4°C. The secondary antibody was <u>ab150177</u> 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.

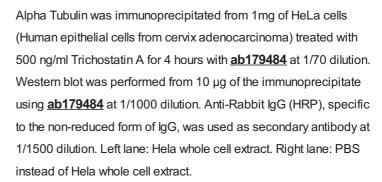
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab179484).

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 hours labeling alpha Tubulin (acetyl K40) with ab179484 at 1/240 dilution (red line). Goat anti rabbit lgG (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 was Rabbit monoclonal lgG (black) and the unlabelled contol was cells without incubation with primary antibody and secondary antibody (blue).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab179484).

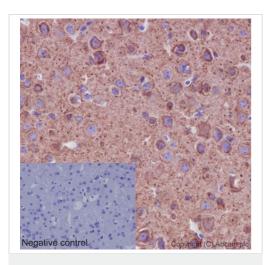


Immunoprecipitation - Anti-alpha Tubulin (acetyl K40) antibody [EPR16772] - BSA and Azide free (ab209348)



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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab179484).



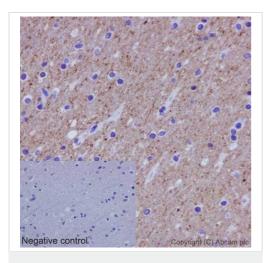
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-alpha Tubulin (acetyl K40) antibody [EPR16772] - BSA and Azide free (ab209348)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab179484).

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



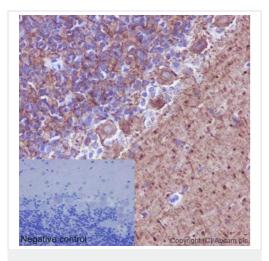
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-alpha Tubulin (acetyl K40) antibody [EPR16772] - BSA and Azide free (ab209348)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab179484</u>).

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



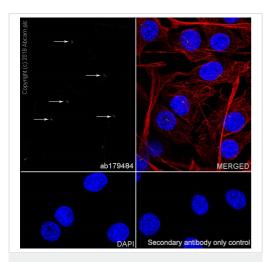
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-alpha Tubulin (acetyl K40) antibody [EPR16772] - BSA and Azide free (ab209348)

This IHC data was generated using the same anti-alpha Tubulin (acetyl K40) antibody clone, EPR16772, in a different buffer formulation (cat# <u>ab179484</u>).

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.

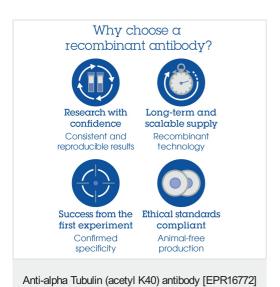
Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Antialpha Tubulin (acetyl K40) antibody [EPR16772] -BSA and Azide free (ab209348) This ICC/IF data was generated using the same anti-alpha Tubulin (acetyl K40) antibody clone, EPR16772, in a different buffer formulation (cat# <u>ab179484</u>).

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.



- BSA and Azide free (ab209348)

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