abcam

Product datasheet

Anti-Tau (phospho S396) antibody [EPR2731] - BSA and Azide free ab156623

Recombinant RabMAb

12 Images

Overview

Product name	Anti-Tau (phospho S396) antibody [EPR2731] - BSA and Azide free
Description	Rabbit monoclonal [EPR2731] to Tau (phospho S396) - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** WB, IP, Dot blot, IHC-P, IHC-Fr **Unsuitable for:** ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: SH-SY5Y treated with alkaline phosphatase, Human and Mouse brain tissue lysate; IHC-P: human glioblastoma, human Alzheimer hippocampus, human, mouse and rat colon; IP- Human brain lysate; IHC-Fr: Mouse and Rat cerebrum tissue, Hu Alzheimer brain.
General notes	ab156623 is the carrier-free version of ab109390. 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 cell-based 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	Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR2731
Isotype	IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab156623 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		Use at an assay dependent concentration. Predicted molecular weight: 79 kDa.
IP		Use at an assay dependent concentration.
Dot blot		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration.
IHC-Fr		Use at an assay dependent concentration. Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).

Application notes Is unsuitable for ICC/IF.

Target

Function Promotes microtubule assembly and stability, and might be involved in the establishment and maintenance of neuronal polarity. The C-terminus binds axonal microtubules while the N-terminus binds neural plasma membrane components, suggesting that tau functions as a linker protein between both. Axonal polarity is predetermined by tau localization (in the neuronal cell) in the domain of the cell body defined by the centrosome. The short isoforms allow plasticity of the cytoskeleton whereas the longer isoforms may preferentially play a role in its stabilization.

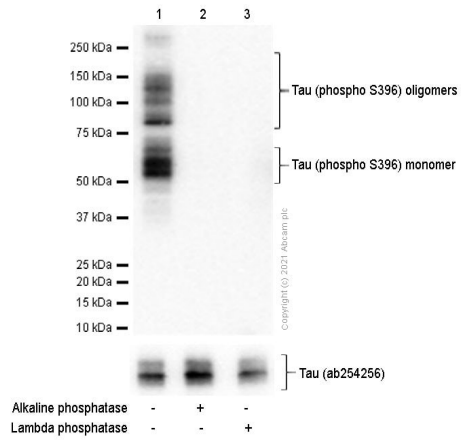
Tissue specificity Expressed in neurons. Isoform PNS-tau is expressed in the peripheral nervous system while the others are expressed in the central nervous system.

Involvement in disease Note=In Alzheimer disease, the neuronal cytoskeleton in the brain is progressively disrupted and

	replaced by tangles of paired helical filaments (PHF) and straight filaments, mainly composed of hyperphosphorylated forms of TAU (PHF-TAU or AD P-TAU). Defects in MAPT are a cause of frontotemporal dementia (FTD) [MIM:600274]; also called frontotemporal dementia (FTD), pallido-ponto-nigral degeneration (PPND) or historically termed Pick complex. This form of frontotemporal dementia is characterized by presenile dementia with behavioral changes, deterioration of cognitive capacities and loss of memory. In some cases, parkinsonian symptoms are prominent. Neuropathological changes include frontotemporal atrophy often associated with atrophy of the basal ganglia, substantia nigra, amygdala. In most cases, protein tau deposits are found in glial cells and/or neurons. Defects in MAPT are a cause of Pick disease of the brain (PIDB) [MIM:172700]. It is a rare form of dementia pathologically defined by severe atrophy, neuronal loss and gliosis. It is characterized by the occurrence of tau-positive inclusions, swollen neurons (Pick cells) and argentophilic neuronal inclusions known as Pick bodies that disproportionally affect the frontal and temporal cortical regions. Clinical features include aphasia, apraxia, confusion, anomia, memory loss and personality deterioration. Note=Defects in MAPT are a cause of corticobasal degeneration (CBD). It is marked by extrapyramidal signs and apraxia and can be associated with memory loss. Neuropathologic features may overlap Alzheimer disease, progressive supranuclear palsy, and Parkinson disease. Defects in MAPT are a cause of progressive supranuclear palsy type 1 (PSNP1) [MIM:601104, 260540]; also abbreviated as PSP and also known as Steele-Richardson-Olszewski syndrome. PSNP1 is characterized by akinetic-rigid syndrome, supranuclear gaze palsy, pyramidal tract dysfunction, pseudobulbar signs and cognitive capacities deterioration. Neurofibrillary tangles and gliosis but no amyloid plaques are found in diseased brains. Most cases appear to be sporadic, with a significant association with a common haplotype including the MAPT gene and the flanking regions. Familial cases show an autosomal dominant pattern of transmission with incomplete penetrance; genetic analysis of a few cases showed the occurrence of tau mutations, including a deletion of Asn-613.
Sequence similarities	Contains 4 Tau/MAP repeats.
Developmental stage	Four-repeat (type II) tau is expressed in an adult-specific manner and is not found in fetal brain, whereas three-repeat (type I) tau is found in both adult and fetal brain.
Domain	The tau/MAP repeat binds to tubulin. Type I isoforms contain 3 repeats while type II isoforms contain 4 repeats.
Post-translational modifications	Phosphorylation at serine and threonine residues in S-P or T-P motifs by proline-directed protein kinases (PDPK: CDK1, CDK5, GSK-3, MAPK) (only 2-3 sites per protein in interphase, seven-fold increase in mitosis, and in PHF-tau), and at serine residues in K-X-G-S motifs by MAP/microtubule affinity-regulating kinase (MARK) in Alzheimer diseased brains. Phosphorylation decreases with age. Phosphorylation within tau's repeat domain or in flanking regions seems to reduce tau's interaction with, respectively, microtubules or plasma membrane components. Phosphorylation on Ser-610, Ser-622, Ser-641 and Ser-673 in several isoforms during mitosis. Polyubiquitinated. Requires functional TRAF6 and may provoke SQSTM1-dependent degradation by the proteasome (By similarity). PHF-tau can be modified by three different forms of polyubiquitination. 'Lys-48'-linked polyubiquitination is the major form, 'Lys-6'-linked and 'Lys-11'-linked polyubiquitination also occur. Glycation of PHF-tau, but not normal brain tau. Glycation is a non-enzymatic post-translational modification that involves a covalent linkage between a sugar and an amino group of a protein molecule forming ketoamine. Subsequent oxidation, fragmentation and/or cross-linking of ketoamine leads to the production of advanced glycation endproducts (AGES). Glycation may play a role in stabilizing PHF aggregation leading to tangle formation in AD.

Cellular localization	Cytoplasm > cytosol. Cell membrane. Cytoplasm > cytoskeleton. Cell projection > axon. Mostly found in the axons of neurons, in the cytosol and in association with plasma membrane components.
Form	There are 9 isoforms produced by alternative splicing.

Images



Western blot - Anti-Tau (phospho S396) antibody [EPR2731] - BSA and Azide free (ab156623)

All lanes : Anti-Tau (phospho S396) antibody [EPR2731] (ab109390) at 1/1000 dilution

Lane 1 : Human brain lysate
Lane 2 : Human brain lysates and the membrane was incubated with alkaline phosphatase
Lane 3 : Human brain lysates and the membrane was incubated with lambda phosphatase

Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

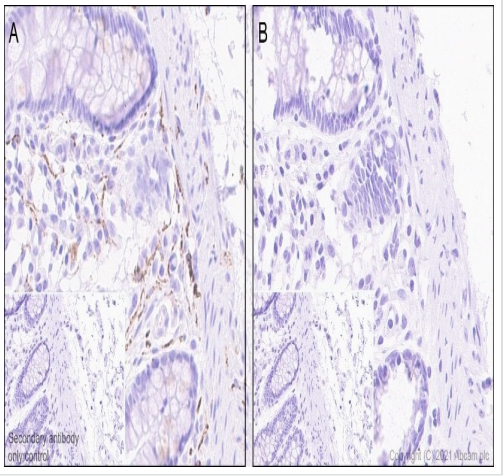
Predicted band size: 79 kDa
Observed band size: 50-79 kDa

Exposure time: 100 seconds

Blocking/Diluting buffer and concentration 5% NFDM/TBST

Tau assembles into oligomers as described in PMID: 28382304, 32692785 and 30120733.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109390).

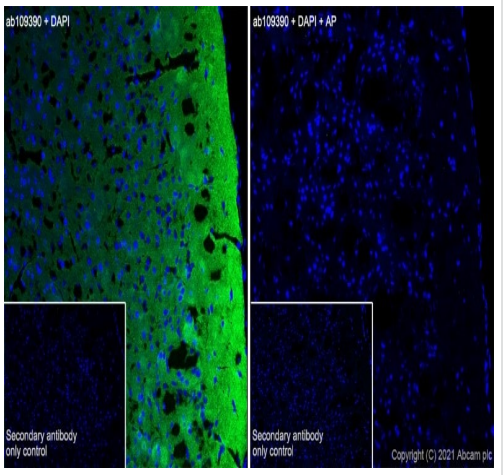


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Tau (phospho S396) antibody [EPR2731] - BSA and Azide free (ab156623)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109390).

Immunohistochemistry analysis of paraffin-embedded human colon tissue sections labeling Tau (phospho S396) with ab109390 at 1/4000 dilution (0.027 µg/mL). Goat Anti-Rabbit IgG H&L (HRP polymer) was used as the secondary antibody. Sections were counterstained with Hematoxylin. Antigen retrieval was heat mediated using ab93684 (Tris/EDTA buffer, pH 9.0).

Positive staining on ganglions of human colon without alkaline phosphatase treatment (image A). No staining on ganglions of human colon with alkaline phosphatase treatment (image B). The section was incubated with ab109390 overnight at +4°C.

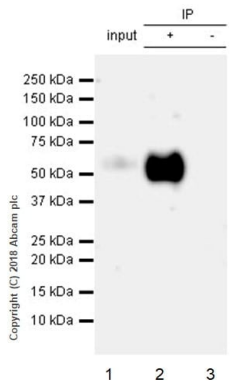


Immunohistochemistry (Frozen sections) - Anti-Tau (phospho S396) antibody [EPR2731] - BSA and Azide free (ab156623)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109390).

Immunohistochemistry analysis of frozen mouse cerebrum tissue sections labeling Tau (phospho S396) with ab109390 at 1/100 (1 µg/mL). ab150077 AlexaFluor®488 Goat anti-Rabbit at 1/1000 (2 µg/mL) was used as the secondary antibody. Sections were fixed with 4% PFA and permeabilised with 0.2% Triton X-100. DAPI (blue) was used as nuclear counterstain. Antigen retrieval was heat mediated using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).

Cytoplasmic staining on mouse cerebrum, the signal decreased after phosphatase treatment at 37▌ for 2h.



Immunoprecipitation - Anti-Tau (phospho S396) antibody [EPR2731] - BSA and Azide free (ab156623)

ab109390 at 1/20 immunoprecipitating Tau (phospho S396) in Human brain lysate.

Lane 1 (input): Human brain lysate (10µg)

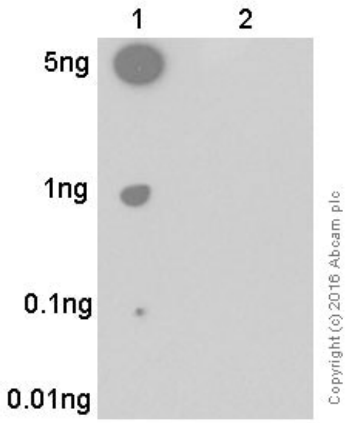
Lane 2 (+): ab109390 + Human brain lysate.

Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab109390 in Human brain lysate.

For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/5000 dilution.

Diluting / Blocking 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 (ab109390).



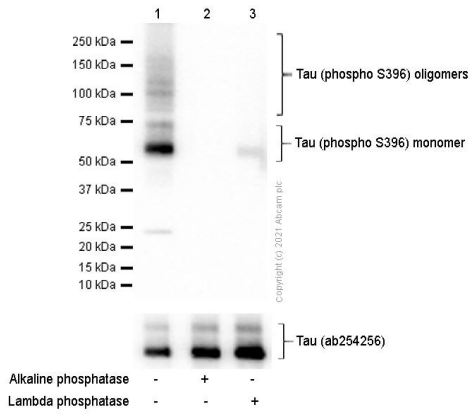
Dot Blot - Anti-Tau (phospho S396) antibody [EPR2731] - BSA and Azide free (ab156623)

Dot blot analysis of Tau (phospho S396) phospho peptide (Lane 1) and Tau non-phospho peptide (Lane 2) labeling Tau (phospho S396) with ab109390 at a dilution of 1/1000. ab97051 (Peroxidase conjugated goat anti-rabbit IgG) (H+L) at 1/100 000 was used as the secondary antibody.

Blocking and diluting buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109390).



Western blot - Anti-Tau (phospho S396) antibody [EPR2731] - BSA and Azide free (ab156623)

All lanes : Anti-Tau (phospho S396) antibody [EPR2731] (ab109390) at 1/1000 dilution

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Lane 3 : Mouse brain lysates and the membrane was incubated with lambda phosphatase 15 µg

Lysates/proteins at 15 µg per lane.

Secondary

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Exposure time: 10 seconds

Diluting/Diluting buffer and concentration 5% NFDM/TBST

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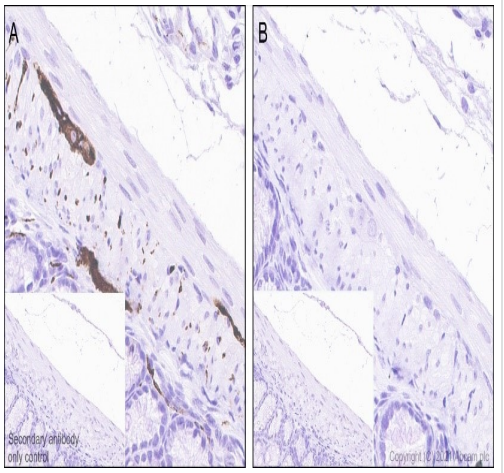
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109390).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Tau (phospho S396) antibody [EPR2731] - BSA and Azide free (ab156623)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109390).

Immunohistochemistry analysis of paraffin-embedded mouse colon tissue sections labeling Tau (phospho S396) with ab109390 at 1/4000 dilution (0.027 µg/mL). Goat Anti-Rabbit IgG H&L (HRP polymer) was used as the secondary antibody. Sections were counterstained with Hematoxylin. Antigen retrieval was heat mediated using ab93684 (Tris/EDTA buffer, pH 9.0).

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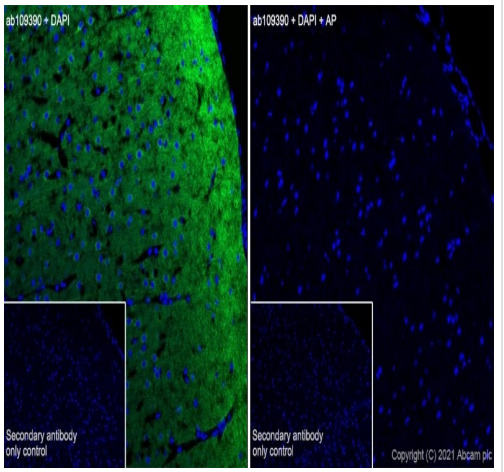
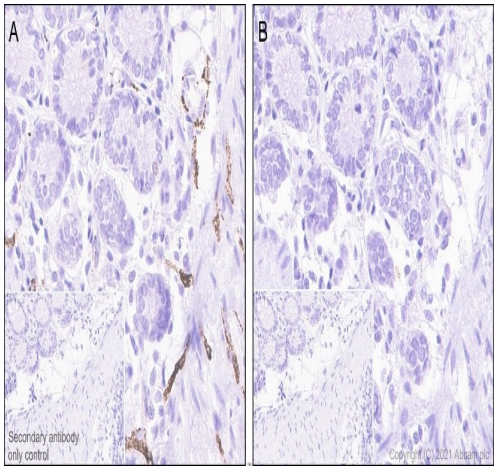


Immunohistochemistry (Frozen sections) - Anti-Tau (phospho S396) antibody [EPR2731] - BSA and Azide free (ab156623)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109390).

Immunohistochemistry analysis of frozen rat cerebrum tissue sections labeling Tau (phospho S396) with ab109390 at 1/100 (1 µg/mL). ab150077 AlexaFluor®488 Goat anti-Rabbit at 1/1000 (2 µg/mL) was used as the secondary antibody. Sections were fixed with 4% PFA and permeabilised with 0.2% Triton X-100. DAPI (blue) was used as nuclear counterstain. Antigen retrieval was heat mediated using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).

Cytoplasmic staining on rat cerebrum, the signal decreased after phosphatase treatment at 37I for 2h.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Tau (phospho S396) antibody [EPR2731] - BSA and Azide free (ab156623)

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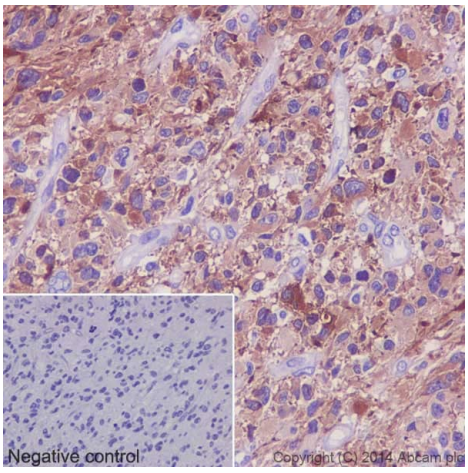
Immunohistochemistry analysis of paraffin-embedded rat colon tissue sections labeling Tau (phospho S396) with ab109390 at 1/4000 dilution (0.027 µg/mL). Goat Anti-Rabbit IgG H&L (HRP polymer) was used as the secondary antibody. Sections were counterstained with Hematoxylin. Antigen retrieval was heat mediated using ab93684 (Tris/EDTA buffer, pH 9.0).

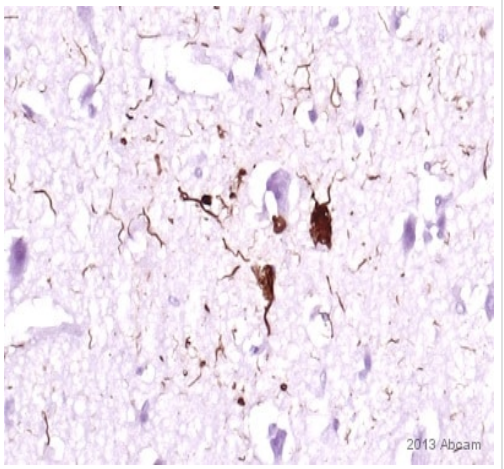
Positive staining on ganglions of rat colon without alkaline phosphatase treatment (image A). No staining on ganglions of rat colon with alkaline phosphatase treatment (image B).
The section was incubated with ab109390 overnight at +4°C.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Tau (phospho S396) antibody [EPR2731] - BSA and Azide free (ab156623)

Immunohistochemical staining of paraffin embedded human glioblastoma with purified ab109390 at a dilution of 1/4000. A pre-diluted HRP polymer for rabbit/mouse IgG was used as the secondary antibody and the sample was counter-stained wirh hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109390).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Tau (phospho S396) antibody [EPR2731] - BSA and Azide free (ab156623)

This image is courtesy of an Abreview submitted by Carl Hobbs.

IHC image of Tau (phospho S396) staining in human Alzheimer hippocampus formalin fixed paraffin embedded tissue section. The section was pre-treated using heat mediated antigen retrieval with citrate buffer. The section was then incubated with unpurified ab109390 at 1/1000 dilution for 2 hoursat 21ºC. A biotin conjugated goat-anti-rabbit antibody was used as a secondary at 1/250. The section shows clear neurofibrillary tangles in a subset of neurons.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109390).

Anti-Tau (phospho S396) antibody [EPR2731] - BSA and Azide free (ab156623)

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