# abcam

## Product datasheet

## Anti-Tau (phospho S396) antibody [EPR2731] ab109390

Recombinant RabMAb

★★★★ 13 Abreviews 110 References

12 Images

#### Overview

**Product name** Anti-Tau (phospho S396) antibody [EPR2731]

**Description** Rabbit monoclonal [EPR2731] to Tau (phospho S396)

**Host species** Rabbit

Specificity The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for

mouse and rat.

The specificity of this antibody refers to P10636-8.

Suitable for: Dot blot, IHC-Fr, IHC-P, WB, IP **Tested applications** 

Unsuitable for: ICC/IF

Species reactivity Reacts with: Mouse, Rat, Human

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

(Peptide available as ab226770)

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 Tau is a protein associated with several disease states, known collectively as tauopathies. The

> most well-known of these is Alzheimer's disease (AD), were tau exhibiting excessive phosphorylation, aggregating to form neurofibrillary tangles. The epitope defined by

phosphorylation of S396 in tau is strongly implicated in AD-associated tau pathology, providing a

valuable target for the development of therapeutic antibodies to capture tau and prevent

spreading of tau pathology.

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** 

1

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Stable for 12 months at -20°C.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 40% Glycerol, 59% PBS, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal
Clone number EPR2731

**Isotype** IgG

#### **Applications**

The Abpromise guarantee Our Abpromise guarantee covers the use of ab109390 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
Dot blot		1/1000.
IHC-Fr	**** <u>(2)</u>	1/100. Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20)
IHC-P	<b>★★★★ (5)</b>	1/4000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat.
WB	**** <u>(3)</u>	1/10000 - 1/50000. Predicted molecular weight: 79 kDa.Can be blocked with <u>Human Tau (phospho S396) peptide</u> (ab226770). For unpurified, use 1/10000 - 1/50000.
IP		1/10 - 1/100.

**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 presentle 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

**Developmental stage** 

**Domain** 

Post-translational modifications

Contains 4 Tau/MAP repeats.

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.

The tau/MAP repeat binds to tubulin. Type I isoforms contain 3 repeats while type II isoforms contain 4 repeats.

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, sevenfold 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.

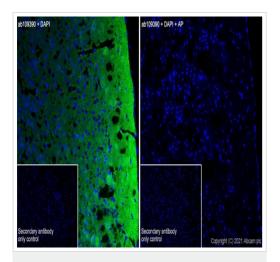
#### **Cellular localization**

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.

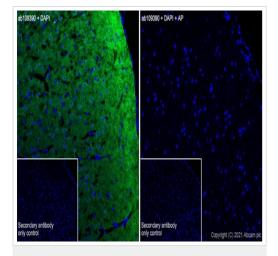
#### **Images**



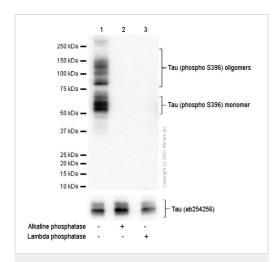
Immunohistochemistry (Frozen sections) - Anti-Tau (phospho S396) antibody [EPR2731] (ab109390)

Immunohistochemistry analysis of frozen mouse cerebrum tissue sections labeling Tau (phospho S396) with ab109390 at 1/100 (1  $\mu$ g/mL). **ab150077** AlexaFluor<sup>®</sup>488 Goat anti-Rabbit at 1/1000 (2  $\mu$ g/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**I** for 2h.



Immunohistochemistry (Frozen sections) - Anti-Tau (phospho S396) antibody [EPR2731] (ab109390)



Western blot - Anti-Tau (phospho S396) antibody [EPR2731] (ab109390)

**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**

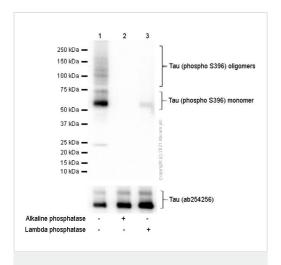
 $\begin{tabular}{ll} \textbf{All lanes:} Goat Anti-Rabbit \ lgG (HRP) with minimal cross-reactivity with human \ lgG \ at 1/2000 \ dilution \end{tabular}$ 

**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.



Western blot - Anti-Tau (phospho S396) antibody [EPR2731] (ab109390)

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

Lane 1: Mouse brain lysate

Lane 2: Mouse brain lysates and the membrane was incubated

with alkaline phosphatase

Lane 3: Mouse 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: 10 seconds

Diluting/Diluting buffer and concentration 5% NFDM/TBST

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

**All lanes :** Anti-Tau (phospho S396) antibody [EPR2731] (ab109390) at 1/20000 dilution (purified)

Lane 1: Untreated SH-SY5Y

Lane 2: SH-SY5Y treated with alkaline phosphatase

Lysates/proteins at 10 µg per lane.

Secondary

All lanes: HRP goat ant-rabbit (H+L) at 1/1000 dilution

**Predicted band size:** 79 kDa **Observed band size:** 50-70 kDa

Western blot - Anti-Tau (phospho S396) antibody [EPR2731] (ab109390)

Tau (phospho S396)

beta III Tubulin (ab52901)

KDa

150 — 100 —

50 -

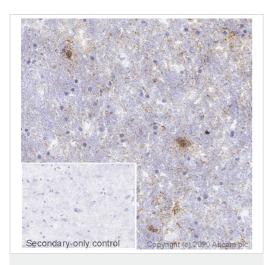
37 -

25 -

alkaline phosphatase:

Blocking buffer: 5% NFDM/TBST

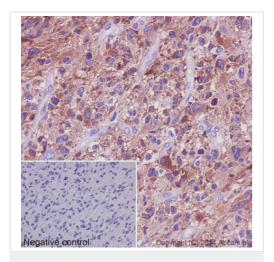
Dilution buffer: 5% NFDM/TBST



Immunohistochemistry (Frozen sections) - Anti-Tau (phospho S396) antibody [EPR2731] (ab109390)

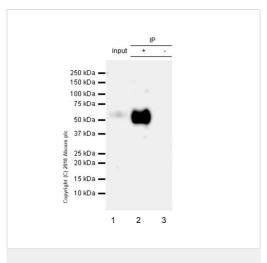
IHC image of Tau (phospho S396) staining in a section of frozen normal human Alzheimer brain performed on a Leica BOND<sup>TM</sup> system using the standard protocol. The section was fixed in 10% paraformaldehyde (10 min) prior to staining. The section was incubated with ab109390, 1/1000 dilution, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Tau (phospho S396) antibody [EPR2731] (ab109390)

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 performed 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.



Immunoprecipitation - Anti-Tau (phospho S396) antibody [EPR2731] (ab109390)

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

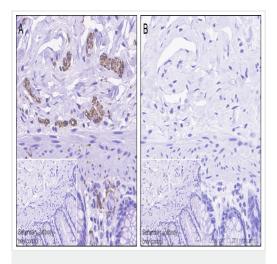
Lane 1 (input): Human brain lysate (10µg)

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

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

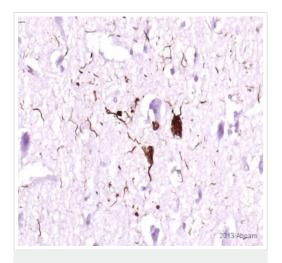
For western blotting, ab109390 at 1/1000 dilution followed by VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/5000 dilution.

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



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Tau (phospho S396) antibody [EPR2731] (ab109390)

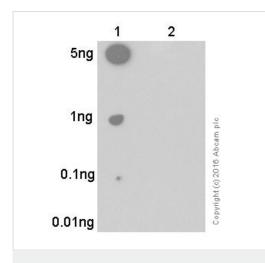
Immunohistochemistry analysis of paraffin-embedded human colon tissue sections labelling Tau (phospho S396) with ab109390 at 1/4000 dilution (0.026 µg/mL). The section was incubated with ab109390 for 30 mins at room temperature. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody. Sections were counterstained with Hematoxylin. Antigen retrieval was heat mediated using Bond ™ Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes. Positive staining on ganglions of human colon without alkaline phosphatase treatment (image A); No signal was detected when tissues were treated with alkaline phosphatase (image B).The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Tau (phospho S396) antibody [EPR2731] (ab109390)

Image courtesy of Carl Hobbs, Kings College London, U.K.

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 21°C. 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.



Dot Blot - Anti-Tau (phospho S396) antibody [EPR2731] (ab109390)

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. <u>ab97051</u> (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.



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