Product datasheet

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

Overview

Product name: Anti-Tau (phospho S396) antibody [EPR2731]
Description: Rabbit monoclonal [EPR2731] to Tau (phospho S396)
Host species: Rabbit
Specificity: This antibody only detects Tau phosphorylated at serine 396.
Tested applications: Suitable for: Dot blot, IHC-P, WB, IP
Unsuitable for: ICC/IF
Species reactivity: Reacts with: Mouse, Rat, Human
Immunogen: Synthetic peptide (the amino acid sequence is considered to be commercially sensitive) corresponding to Human Tau aa 350 to the C-terminus.
Database link: P10636-8
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 +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

Application notes: Is unsuitable for ICC/IF.

Applications

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.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tbody>
<tr>
<td>Dot blot</td>
<td>1/1000.</td>
<td></td>
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<tr>
<td>IHC-P</td>
<td>1/4000.</td>
<td>Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
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<tr>
<td>IP</td>
<td>1/10 - 1/100.</td>
<td></td>
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</table>

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, anoma, 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.

<table>
<thead>
<tr>
<th>Sequence similarities</th>
<th>Contains 4 Tau/MAP repeats.</th>
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<tbody>
<tr>
<td>Developmental stage</td>
<td>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.</td>
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<td>Domain</td>
<td>The tau/MAP repeat binds to tubulin. Type I isoforms contain 3 repeats while type II isoforms contain 4 repeats.</td>
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<tr>
<td>Post-translational modifications</td>
<td>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.</td>
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<td>Form</td>
<td>There are 9 isoforms produced by alternative splicing.</td>
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| Images                | 3 |
**Western blot - Anti-Tau (phospho S396) antibody [EPR2731] (ab109390)**

**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

*why is the actual band size different from the predicted?*

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST

**Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded 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 hours at 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.
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 ab131366 VeriBlot for IP (HRP) was used as the secondary antibody (1/5000).

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

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 with 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.
**Western blot - Anti-Tau (phospho S396) antibody [EPR2731] (ab109390)\(^\text{[EPR2731]}\)**

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

- **Lane 1**: Human brain whole cell lysates.
- **Lane 2**: Human brain whole cell lysates. The membrane was incubated with phosphatase.

Lysates/proteins at 15 µg per lane.

**Secondary**
- **All lanes**: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

**Predicted band size**: 79 kDa

**Exposure time**: 1 minute

**Diluting and blocking buffer**: 5% NFDM/TBST

- Anti-Tau (phospho S396) antibody [EPR2731] (ab109390) at 1/5000 dilution (purified) + Mouse brain at 10 µg

**Secondary**
- HRP goat anti-rabbit (H+L) at 1/1000 dilution

**Predicted band size**: 79 kDa

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

**why is the actual band size different from the predicted?**

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST
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.

All lanes: Anti-Tau (phospho S396) antibody [EPR2731] (ab109390) at 1/10000 dilution (unpurified)

Lane 1: SH-SY5Y cell lysates, untreated
Lane 2: SH-SY5Y cell lysates, treated with Alkaline Phosphatase

Lysates/proteins at 10 µg per lane.

Predicted band size: 79 kDa

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