

Product datasheet

Anti-Tau (phospho S404) antibody [EPR2605] ab92676

Recombinant RabMAb

***** 4 Abreviews 29 References 13 Images

Overview

| Product name | Anti-Tau (phospho S404) antibody [EPR2605] |
|---------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Description | Rabbit monoclonal [EPR2605] to Tau (phospho S404) |
| Host species | Rabbit |
| Specificity | The specificity of this antibody refers to P10636-8. |
| Tested applications | Suitable for: IP, WB, IHC-P, IHC-Fr, ICC/IF, Dot blot |
| Species reactivity | Reacts with: Mouse, Rat, Human |
| Immunogen | Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. |
| Positive control | ICC/IF: Mouse primary cortical cultures; WB: Human, mouse and rat brain lysate; IHC: Mouse and rat cerebrum and Human glioma tissue; IP: Human brain lysate, IHC-Fr: Mouse cerebrum tissue. |
| 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 <u>see here</u>. Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb[®] patents</u>. |

Properties

| Form | Liquid | | |
|-----------------------------------------|------------------------------------------------------------------------|--|--|
| Storage instructions | Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C. | | |
| Dissociation constant (K _D) | $K_{D} = 1.67 \times 10^{-11} M$ | | |
| | LOW 10 ⁻⁶ 10 ⁻¹¹ 10 ⁻¹³ HIGH AFFINITY | | |

Learn more about K_D

Storage buffer

pH: 7.20

| | Preservative: 0.05% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), 59% PBS, 0.01% BSA |
|--------------|----------------------------------------------------------------------------------------------------------|
| Purity | Protein A purified |
| Clonality | Monoclonal |
| Clone number | EPR2605 |
| lsotype | lgG |

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Applications

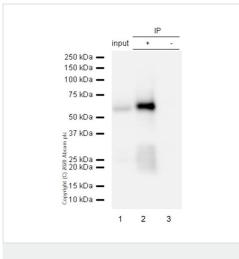
The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab92676 in the following tested applications.

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

| Application | Abreviews | Notes |
|-------------|-------------------------|----------------------------------------------------------------------------------------------------------------|
| IP | | 1/20. |
| WB | ★ ★ ★ ★ ★ <u>(3)</u> | 1/500 - 1/2000. Predicted molecular weight: 79 kDa. |
| IHC-P | | 1/100 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. |
| IHC-Fr | | 1/50. Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20) |
| ICC/IF | ★★★★★ <u>(1)</u> | 1/50. |
| Dot blot | | Use at an assay dependent concentration. |

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, 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. |



Immunoprecipitation - Anti-Tau (phospho S404) antibody [EPR2605] (ab92676)



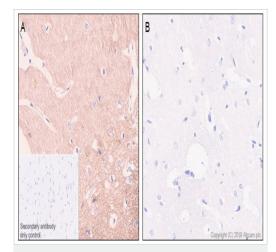
Immunoprecipitation - Anti-Tau (phospho S404) antibody [EPR2605] (ab92676) Purified ab92676 at 1/20 dilution (0.5µg) immunoprecipitating Tau in Mouse brain lysate.
Lane 1 (input): Mouse brain lysate 10µg
Lane 2 (+): ab92676 + Mouse brain lysate.
Lane 3 (-): Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab92676 in Mouse brain lysate.
VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) (1/1000 dilution) was used for Western blotting.
Blocking Buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM/TBST.
Observed band size: 50-70 kDa

Purified ab92676 at 1/20 dilution (0.5µg) immunoprecipitating Tau in Human brain lysate. Lane 1 (input): Human brain lysate 10µg Lane 2 (+): ab92676 + Human brain lysate. Lane 3 (-): Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab92676 in Human brain lysate. VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) (1/5000 dilution) was used for Western blotting.

Blocking Buffer and concentration: 5% NFDM/TBST.

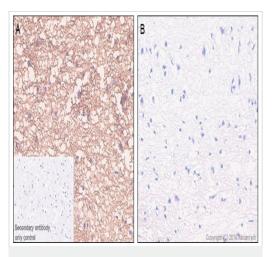
Diluting buffer and concentration: 5% NFDM/TBST.

Observed band size: 50-70 kDa



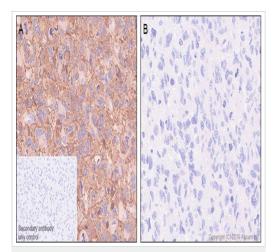
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Rat cerebrum tissue sections labeling Tau with purified ab92676 at 1/200 dilution (0.53 µg/mL). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Tau (phospho S404) antibody [EPR2605] (ab92676)



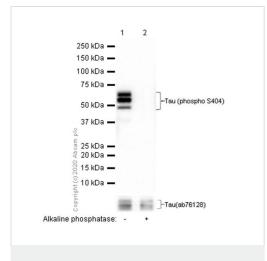
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Tau (phospho S404) antibody [EPR2605] (ab92676)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Mouse cerebrum tissue sections labeling Tau with purified ab92676 at 1/200 dilution (0.53 µg/mL). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.

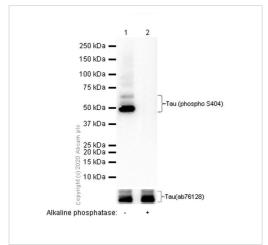


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human glioma tissue sections labeling Tau with purified ab92676 at 1/200 dilution (0.53 µg/mL). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Tau (phospho S404) antibody [EPR2605] (ab92676)



Western blot - Anti-Tau (phospho S404) antibody [EPR2605] (ab92676)



Western blot - Anti-Tau (phospho S404) antibody [EPR2605] (ab92676) **All lanes :** Anti-Tau (phospho S404) antibody [EPR2605] (ab92676) at 1/1000 dilution (Purified)

Lane 1 : Rat brain lysate Lane 2 : Rat brain lysate, the menbrane treated with alkaline phosphatase for 1 hour

Secondary

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

Predicted band size: 79 kDa

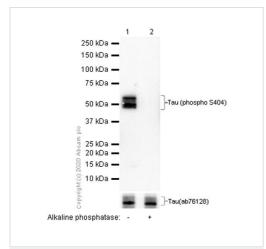
All lanes : Anti-Tau (phospho S404) antibody [EPR2605] (ab92676) at 1/1000 dilution (Purified)

Lane 1 : Mouse brain lysate Lane 2 : Mouse brain lysate, the menbrane treated with alkaline phosphatase for 1 hour

Secondary

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

Predicted band size: 79 kDa



Western blot - Anti-Tau (phospho S404) antibody [EPR2605] (ab92676)

All lanes : Anti-Tau (phospho S404) antibody [EPR2605] (ab92676) at 1/1000 dilution (Purified)

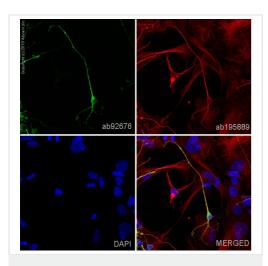
Lane 1 : Human brain lysate

Lane 2 : Human brain lysate, the menbrane treated with alkaline phosphatase for 1 hour

Secondary

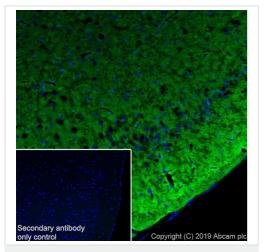
All lanes : Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Predicted band size: 79 kDa



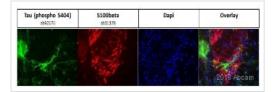
Immunocytochemistry/ Immunofluorescence - Anti-Tau (phospho S404) antibody [EPR2605] (ab92676) Immunocytochemistry/ Immunofluorescence analysis of Embryonic mouse primary neural cells labeling Tau with purified ab92676 at 1:100 dilution (1 μ g/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Goat anti rabbit IgG (Alexa Fluor[®] 488, **ab150077**) was used as the secondary antibody at 1:1000 (2 μ g/ml) dilution. DAPI (blue) was used as nuclear counterstain. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) at 1:200 (2.5 μ g/ml) dilution. DAPI (blue) was used as nuclear counterstain.

Confocal image showing positive staining in neuron.



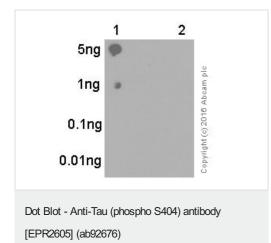
Immunohistochemistry (Frozen sections) - Anti-Tau (phospho S404) antibody [EPR2605] (ab92676)

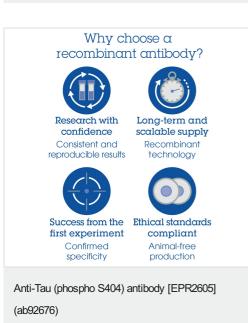
Immunohistochemistry (Frozen sections) analysis of mouse cerebrum tissue sections labeling Tau with Purified ab92676 at 1/50 (1.9 μ g/ml).Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20). Goat anti rabbit lgG (Alexa Fluor[®] 488, **ab150077**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. DAPI was used as a counterstain.



Mouse primary cortical cultures stained for Tau (phospho S404) (green) using ab92676 at 1/100 dilution in ICC/IF, followed by Donkey anti-Rabbit IgG (H+L) Alexa Fluor 488[®].

Immunocytochemistry/ Immunofluorescence - Anti-Tau (phospho S404) antibody [EPR2605] (ab92676) This image is courtesy of an anonymous Abreview.





Dot blot analysis of Tau (pS404) phosopho peptide (Lane 1) and Tau non-phospho peptide (Lane 2) labelling Tau (phospho S404) with ab92676 at a dilution of 1/1000. <u>ab97051</u> (Peroxidase conjugated goat anti-rabbit IgG (H+L)) was used as the secondary antibody at a dilution of 1/100000.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.

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