

Product datasheet

Anti-Tau antibody [EP2456Y] ab76128

Recombinant RabMAb

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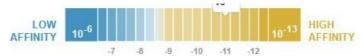
Overview

Product name	Anti-Tau antibody [EP2456Y]
Description	Rabbit monoclonal [EP2456Y] to Tau
Host species	Rabbit
Specificity	This antibody detects Tau, whether phosphorylated or unphosphorylated on serine 635. The specificity of this antibody refers to P10636-8. This antibody clone has been shown to react with Tau 4R and 3R isoforms (doi.org/10.1101/2023.04.13.536711). Our testing suggests that this antibody clone does not cross-reacts with MAP2 or MAP4
Tested applications	Suitable for: Flow Cyt (Intra), ICC/IF, WB Unsuitable for: IP
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as <u>ab211410</u>)
Positive control	ICC/IF: Neuro-2a cells; Flow Cyt (intra): SH-SY5Y cells.
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 see here. 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 +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Dissociation constant (K _D)	$K_D = 1.55 \times 10^{-11} M$

10-11



Learn more about K_D

Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP2456Y
Isotype	lgG

Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab76128 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)		1/500. For unpurified use at 1/160 - 1/1000. <u>ab172730</u> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/50. For unpurified use at 1/100 - 1/500.
WB		1/10000. Detects a band of approximately 50 kDa.Can be blocked with <u>Anti-Tau antibody - Blocking Peptide</u> (ab211410). For unpurified use at 1/2000 - 1/10000.

Application notes

Is unsuitable for IP.

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.
Expressed in neurons. Isoform PNS-tau is expressed in the peripheral nervous system while the others are expressed in the central nervous system.
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 canalysis of a few cases showed the occurrence of tau mutations, including the mathematic canalysis of a few cases showed the occurrence of tau mutations, including the protext of the often
Sequence similarities	including a deletion of Asn-613. 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.

Images



Western blot - Anti-Tau antibody [EP2456Y] (ab76128) All lanes : Anti-Tau antibody [EP2456Y] (ab76128) at 1/1000 dilution

Lane 1 : 293T cells transfected with an empty vector containing a flag tag whole cell lysate
Lane 2 : 293T cells transfected with a human Tau expression

vector containing a flag whole cell lysate

Lane 3 : 293T cells transfected with a human MAP2 expression vector containing a flag whole cell lysate

Lane 4 : 293T cells transfected with a human MAP4 expression vector containing a flag whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution (Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated)

Observed band size: 55-100 kDa

Exposure time: 1 second

Blocking/dilution buffer: 5% NFDM/TBST

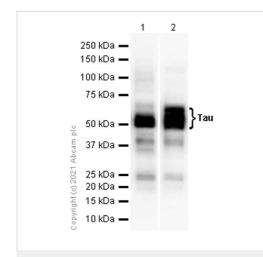
All lanes : Anti-Tau antibody [EP2456Y] (ab76128) at 1/10000 dilution (Purified)

Lane 1 : Mouse brain lysate

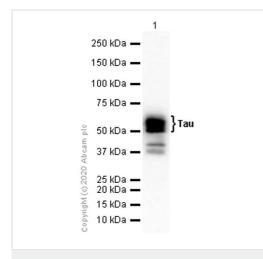
Lane 2 : Rat brain lysate

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution



Western blot - Anti-Tau antibody [EP2456Y] (ab76128)



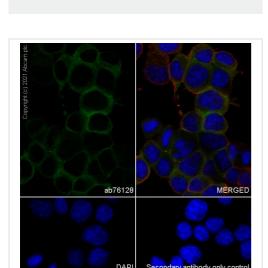
Western blot - Anti-Tau antibody [EP2456Y]

(ab76128)

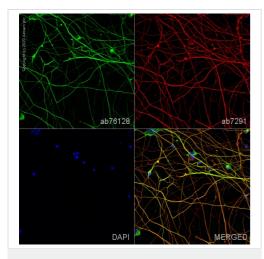
Anti-Tau antibody [EP2456Y] (ab76128) at 1/20000 dilution (Purified) + Human brain lysate

Secondary

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



Immunocytochemistry/ Immunofluorescence - Anti-Tau antibody [EP2456Y] (ab76128) Immunocytochemistry analysis of Neuro-2a (Mouse neuroblastoma neuroblast) cells labeling Tau with purified ab76128 at 1/50 dilution (10 µg/mL). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% TritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) 1/200 (2.5 µg/mL). Goat anti rabbit IgG (Alexa Fluor[®] 488, **ab150077**) was used as the secondary antibody at 1/1000 (2 µg/mL) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

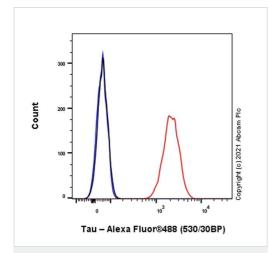


Immunocytochemistry/ Immunofluorescence - Anti-Tau antibody [EP2456Y] (ab76128)

Immunofluorescence staining of Tau using ab76128 (Unpurified format) in ioGlutamatergic Neurons (Human iPSC-Derived Glutamatergic Neurons, <u>ab259259</u>), which were differentiated for 11 days post induction.

The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% PBS-Tween for 5 mins and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab76128 at 0.5 µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin, at 1/1000 dilution. Cells were then incubated with **ab150081**, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Images were acquired with the Perkin Elmer Operetta HCA and a maximum intensity projection of confocal sections is shown.



Flow Cytometry (Intracellular) - Anti-Tau antibody [EP2456Y] (ab76128) Intracellular Flow Cytometry analysis of SH-SY5Y (Human neuroblastoma epithelial cell) cells labelling Tau with purified ab76128 at 1/500 dilution (1 µg/mL) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor[®] 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabelled control - Cell without incubation with primary antibody and secondary antibody (Blue).

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Anti-Tau antibody [EP2456Y] (ab76128)

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