## abcam

#### Product datasheet

### Anti-Tau (phospho T205) antibody [EPR23505-13] - BSA and Azide free ab275027

Recombinant RabMAb

#### 13 Images

#### Overview

**Product name** Anti-Tau (phospho T205) antibody [EPR23505-13] - BSA and Azide free

**Description** Rabbit monoclonal [EPR23505-13] to Tau (phospho T205) - BSA and Azide free

**Host species** Rabbit

Specificity The specificity of this antibody refers to P10636-8.

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

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: Human brain tissue lysate; Mouse brain tissue lysate; rat hippocampus tissue lysate. IHC-P:

> Human Alzheimer's disease cerebrum and breast tissue; Rat cerebrum tissue. IHC-Fr: Rat cerebrum tissue; Mouse cerebrum tissue. IP: Rat brain tissue lysate; Mouse brain tissue lysate.

General notes ab275027 is the carrier-free version of ab254410.

> 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 cellbased 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<sup>®</sup> Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar<sup>®</sup> 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.

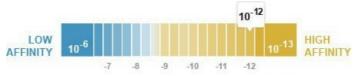
Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**<sup>®</sup> **patents**.

#### **Properties**

Form Liquid

**Storage instructions** Shipped at 4°C. Store at +4°C. Do Not Freeze.

**Dissociation constant (K<sub>D</sub>)**  $K_D = 1.00 \times 10^{-12} M$ 



Learn more about K<sub>D</sub>

Storage buffer Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal
Clone number EPR23505-13

**Isotype** IgG

#### **Applications**

The Abpromise guarantee Our Abpromise guarantee covers the use of ab275027 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. Detects a band of approximately 50-70 kDa (predicted molecular weight: 78 kDa).
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).
Dot blot		Use at an assay dependent concentration.
AP		Use at an assay dependent concentration. <b>Antibody concentration range -</b> 6.67, 3.33, 1.67, 0.83, 0.42, 0 nM/mL
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

#### **Target**

#### **Function**

### Tissue specificity

#### Involvement in disease

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 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, 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.

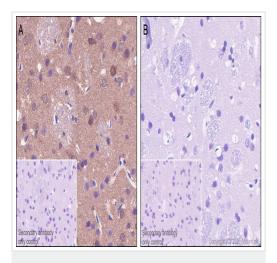
Cytoplasm > cytosol. Cell membrane. Cytoplasm > cytoskeleton. Cell projection > axon. Mostly found in the axons of neurons, in the cytosol and in association with plasma membrane components.

There are 9 isoforms produced by alternative splicing.

#### **Cellular localization**

**Form** 

#### **Images**



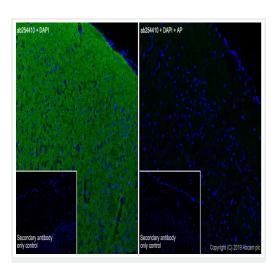
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Tau (phospho T205) antibody [EPR23505-13] - BSA and Azide free (ab275027)

This data was developed using <u>ab254410</u>, the same antibody clone in a different buffer formulation.

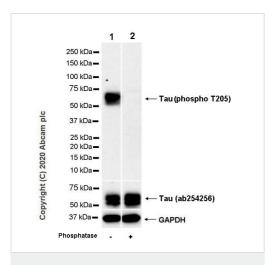
Immunohistochemical analysis of paraffin-embedded Rat cerebrum tissue labeling Tau (phospho T205) with <u>ab254410</u> at 1/20000 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>). Positive staining on rat cerebrum without alkaline phosphatase treatment (image A, PMID: 28035925). No signal was detected when tissues were treated with alkaline phosphatase (image B). The section was incubated with <u>ab254410</u> for 10 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND<sup>®</sup> RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 10 mins.



Immunohistochemistry (Frozen sections) - Anti-Tau (phospho T205) antibody [EPR23505-13] - BSA and Azide free (ab275027)



Western blot - Anti-Tau (phospho T205) antibody [EPR23505-13] - BSA and Azide free (ab275027)

This data was developed using <u>ab254410</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Mouse cerebrum tissue labeling Tau (phospho T205) with <a href="mailto:ab254410">ab254410</a> at 1/500 (1.034 ug/ml) dilution followed by <a href="mailto:ab5454410">ab150077</a> Goat Anti-Rabbit lgG H&L (Alexa Fluor<sup>®</sup> 488) at 1/1000 dilution (Green). Positive staining on mouse cerebrum, while nearly no staining on mouse cerebrum after alkaline phosphatase (AP) treatment. The nuclear counterstain was DAPI (Blue).

Secondary antibody control: Secondary antibody is <u>ab150077</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor<sup>®</sup> 488) at 1/1000 dilution.

Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).

**All lanes**: Anti-Tau (phospho T205) antibody [EPR23505-13] (ab254410) at 1/1000 dilution

Lane 1: Human brain tissue lysate

**Lane 2 :** Human brain tissue lysate (phosphatase treated membrane)

Lysates/proteins at 10 µg per lane.

#### **Secondary**

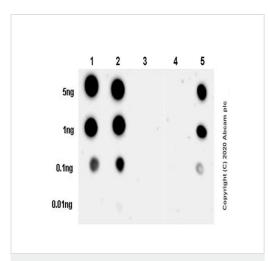
**All lanes :** Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated (ab97051) at 1/100000 dilution

**Predicted band size:** 78 kDa **Observed band size:** 50-70 kDa

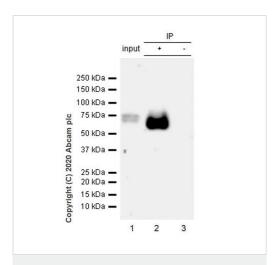
This data was developed using <u>ab254410</u>, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.



Dot Blot - Anti-Tau (phospho T205) antibody [EPR23505-13] - BSA and Azide free (ab275027)



Immunoprecipitation - Anti-Tau (phospho T205) antibody [EPR23505-13] - BSA and Azide free (ab275027)

The molecular weight observed is consistent with what has been described in the literature (PMID: 21722209).

This data was developed using <u>ab254410</u>, the same antibody clone in a different buffer formulation.

Dot blot analysis of Tau (phospho T205) labeled with  $\underline{ab254410}$  at 1/1000 dilution.

Lane 1: Tau (phospho S202+T205) peptide (aa 199-211).

Lane 2: Tau (phospho S202+T205) peptide (aa 197-209).

Lane 3: Tau peptide (aa 197-211).

Lane 4: Tau (phospho S202) peptide (aa 197-211).

Lane 5: Tau (phospho T205) peptide (aa 197-211).

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution was used as secondary antibody.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.

This data was developed using <u>ab254410</u>, the same antibody clone in a different buffer formulation.

Tau (phospho T205) was immunoprecipitated from 0.35 mg Rat brain tissue lysate with <u>ab254410</u> at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using <u>ab254410</u> at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP)(<u>ab131366</u>) was used at 1/5000 dilution.

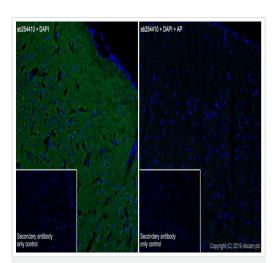
Lane 1: Rat brain tissue lysate 10 ug

Lane 2: ab254410 IP in Rat brain tissue lysate

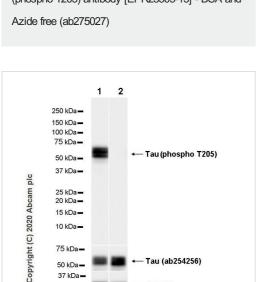
**Lane 3:** Rabbit monoclonal  $\lg G$  (<u>ab172730</u>) instead of <u>ab254410</u> in Rat brain tissue lysate

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

Exposure time: 3 minutes.



Immunohistochemistry (Frozen sections) - Anti-Tau (phospho T205) antibody [EPR23505-13] - BSA and



Western blot - Anti-Tau (phospho T205) antibody [EPR23505-13] - BSA and Azide free (ab275027)

37 kDa=

Tau (ab254256)

GAPDH

This data was developed using ab254410, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Rat cerebrum tissue labeling Tau (phospho T205) with ab254410 at 1/500 (1.034 ug/ml) dilution followed by ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution (Green). Positive staining on rat cerebrum, while nearly no staining on rat cerebrum after alkaline phosphatase (AP) treatment. The nuclear counterstain was DAPI (Blue).

Secondary antibody control: Secondary antibody is ab150077 Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488)at 1/1000 dilution.

Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).

All lanes: Anti-Tau (phospho T205) antibody [EPR23505-13] (ab254410) at 1/1000 dilution

Lane 1: Rat hippocampus tissue lysate

Lane 2: Rat hippocampus tissue lysate (phosphatase treated membrane)

Lysates/proteins at 10 µg per lane.

#### Secondary

All lanes: Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) at 1/100000 dilution

Predicted band size: 78 kDa Observed band size: 50-70 kDa

This data was developed using ab254410, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 48 seconds.

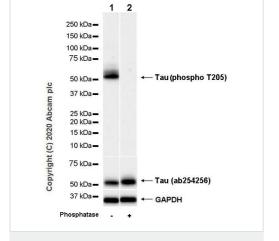
The molecular weight observed is consistent with what has been described in the literature (PMID: 21722209).

**All lanes :** Anti-Tau (phospho T205) antibody [EPR23505-13] (ab254410) at 1/1000 dilution

Lane 1: Mouse brain tissue lysate

**Lane 2 :** Mouse brain tissue lysate (phosphatase treated membrane)

Lysates/proteins at 10 µg per lane.



Western blot - Anti-Tau (phospho T205) antibody [EPR23505-13] - BSA and Azide free (ab275027)

#### Secondary

**All lanes :** Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated (ab97051) at 1/100000 dilution

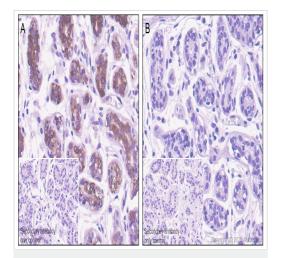
**Predicted band size:** 78 kDa **Observed band size:** 50-70 kDa

This data was developed using <u>ab254410</u>, the same antibody clone in a different buffer formulation.

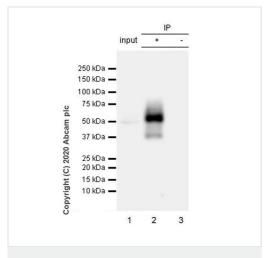
Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 70 seconds.

The molecular weight observed is consistent with what has been described in the literature (PMID: 21722209).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Tau (phospho T205) antibody [EPR23505-13] - BSA and Azide free (ab275027)



Immunoprecipitation - Anti-Tau (phospho T205) antibody [EPR23505-13] - BSA and Azide free (ab275027)

This data was developed using <u>ab254410</u>, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Human breast tissue labeling Tau (phospho T205) with <u>ab254410</u> at 1/20000 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>). Positive staining on human breast without alkaline phosphatase treatment (image A, PMID: 15914550). No signal was detected when tissues were treated with alkaline phosphatase (image B). The section was incubated with <u>ab254410</u> for 10 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND<sup>®</sup> RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 10 mins.

This data was developed using <u>ab254410</u>, the same antibody clone in a different buffer formulation.

Tau (phospho T205) was immunoprecipitated from 0.35 mg Mouse brain tissue lysate with <u>ab254410</u> at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using <u>ab254410</u> at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP)(<u>ab131366</u>) was used at 1/5000 dilution.

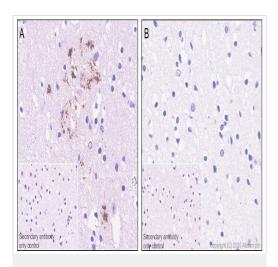
Lane 1: Mouse brain tissue lysate 10 ug

Lane 2: ab254410 IP in Mouse brain tissue lysate

**Lane 3:** Rabbit monoclonal  $\lg G$  (<u>ab172730</u>) instead of <u>ab254410</u> in Mouse brain tissue lysate

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

Exposure time: 10 seconds.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Tau (phospho T205) antibody [EPR23505-13] - BSA and Azide free (ab275027)

This data was developed using <u>ab254410</u>, the same antibody clone in a different buffer formulation.

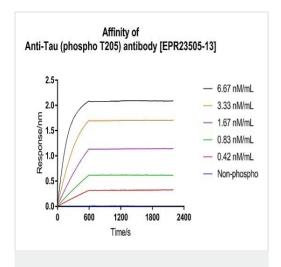
Immunohistochemical analysis of paraffin-embedded Human Alzheimer's disease cerebrum tissue labeling Tau (phospho T205) with <a href="mailto:ab254410">ab254410</a> at 1/20000 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (<a href="mailto:ab209101">ab209101</a>). Positive staining on human Alzheimer's disease cerebrum without alkaline phosphatase treatment (image A, PMID: 20631843). No signal was detected when tissues were treated with alkaline phosphatase (image B). The section was incubated with <a href="mailto:ab254410">ab254410</a> for 10 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

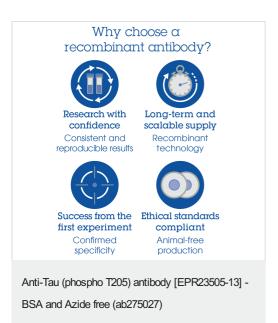
Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 10 mins.

Biotinylated Tau (phospho T205) peptide [0.1 μg/ml] was loaded to SA biosensor on Fortebio RED96e Machine, then associate with recombinant Anti-Tau (phospho T205) antibody [EPR23505-13] in serial concentration points [6.67, 3.33, 1.67, 0.83, 0.42 nM/mL] by 2-fold dilution, next to dissociate in blank testing buffer [0.1% BSA in PBST (0.05%Tween-20)]. Calculated signals had already subtracted blank control, curve fitting using 1:1 binding model. Non-phospho Tau peptide' association and dissociation were also showed in graph. KD(M) value of Anti-Tau (phospho T205) antibody [EPR23505-13] is <1.0E-12

This data was developed using <u>ab254410</u>, the same antibody clone in a different buffer formulation.



Affinity Purification - Anti-Tau (phospho T205) antibody [EPR23505-13] - BSA and Azide free (ab275027)



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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