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Product Information

Anti-phospho-Tau (pSer^{396})
produced in rabbit, affinity isolated antibody

Product Number **T7319**

Product Description

Anti-phospho-Tau (pSer^{396}) is produced in rabbit using as immunogen a synthetic phosphopeptide derived from the region of tau that contains serine 396. The serum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity towards a non-phosphorylated tau.

Anti-phospho-Tau (pSer^{396}) recognizes human, mouse and rat tau (pSer^{396}) (45-68 kDa). It has been used in immunoblotting applications.

Tau is a microtubule-associated phosphoprotein (MAP), localized in neuronal axons. It promotes tubulin polymerization and stabilizes microtubules.[1] The biological activity of tau is regulated by its degree of phosphorylation.[1,2] Hyperphosphorylated tau is the major protein of the paired helical filaments (PHFs), which make up the pathological neurofibrillary tangles of Alzheimer's disease (AD). The PHFs are also found in the lesions of other central nervous system disorders.[3,4]

Tau phosphorylation involves numerous kinases: glycogen synthase kinase 3β (GSK-3β), MARK kinase, MAP kinase, protein kinase A and C, cyclin-dependent kinase 5 (Cdk5), p38 kinase, c-Jun N-terminal kinase, and casein kinase II.[1,2,5,6,7] Combined tau protein kinase II (TPKII), which consists of Cdk5 and GSK-3β, is the most potent phosphorylation agent indirectly involved in the regulation of the phosphorylation state of tau in neuronal cells.[6,8] In addition, tau is phosphorylated *in vitro* by osmotic cellular stress, which activates the stress-activated protein kinases (SAPKs).

To date, a total of 25 abnormal phosphorylation sites have been identified on hyperphosphorylated tau in AD brain.[10] Normal tau has approximately eight phosphorylation sites. The abnormal phosphorylation occurs usually on serine and threonine residues. Specifically, TPKII phosphorylates serines 202 and 404. GSK-3β transfection phosphorylates serines 199, 202, 235, 396, 404 and 413, and threonines 205 and 231. These sites are among the major abnormal phosphorylation sites of tau.[11] Phosphorylation on these sites reduces the ability of a given tau species to promote microtubule self-assembly.[11,12] Okadaic acid increases phosphorylation at threonine 231 and serines 235, 396 and 404. Phosphorylated serine 422 was found in the biopsies of brains from patients with Down syndrome, amyotropic lateral sclerosis, corticobasal degeneration, and Pick's disease. It was absent from a control group of normal brains.[13]

The opposite process, tau dephosphorylation, is controlled by different protein phosphatases expressed in neurons. Protein phosphatases PP2A and PP2B efficiently dephosphorylate tau *in vitro* and restore biological activity in the assembly of microtubules.[3,10,14]

It was discovered that propyl isomerase (Pin 1) interacts with tau hyperphosphorylated on threonine 231 and restores the ability of tau to bind to microtubules.

Reagent

Supplied as a solution in Dulbecco's PBS, pH 7.3, with 1 mg/ml BSA, 50% glycerol, and 0.05% sodium azide.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store at −20 °C. For extended storage, upon initial thawing, freeze in working aliquots. Avoid repeated freezing and thawing to prevent denaturing the antibody. Working dilution samples should be discarded if not used within 12 hours.

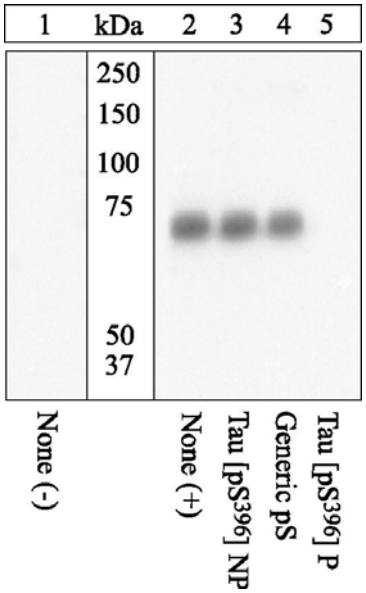
Product Profile

Immunoblotting: a recommended working dilution is 1:1,000

Results

Peptide Competition

1. Human recombinant tau added to background extracts left untreated (1) or treated with GSK-3β (2-5) were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF.
2. Membranes were blocked with a 5% BSA-TBST buffer for 1 hour at room temperature.
3. After blocking, membranes were preincubated with different peptides as follow:
   - Lane 1, 2 no peptide
   - Lane 3 non phosphorylated peptide corresponding to the immunogen
   - Lane 4 a generic phosphoserine containing peptide
   - Lane 5 the phosphopeptide immunogen
4. After preincubation membranes were incubated with Anti-phospho-Tau [pSer^{396}] antibody in a 1% BSA-TBST buffer for two hours at room temperature.
5. After washing, membranes were incubated with goat F(ab')$_2$ IgG-HRP conjugate and bands were detected using the Pierce SuperSignal® method.



The results show that only the peptide corresponding to phospho-Tau [pSer^{396}] blocks the antibody signal, thereby demonstrating the specificity of the antibody.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

References

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AI,PHC 07/16-1