

## Product Information

### Anti-phospho-Tau (pSer<sup>199</sup>)

Developed in Rabbit, Affinity Isolated Antibody

Product Number **T 8444**

#### Product Description

Anti-Phospho-Tau (pSer<sup>199</sup>) is developed in rabbit using a synthetic phosphopeptide derived from the region of human tau that contains serine<sup>199</sup> as immunogen. The serum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity toward non-phosphorylated tau.

Anti-Phospho-Tau (pSer<sup>199</sup>) recognizes human tau (pSer<sup>199</sup>) (60 kDa) by immunoblotting. Mouse and rat tau (100% homologous) have not been tested but are expected to react.

Tau is a microtubule-associated phosphoprotein (MAPs), localized in neuronal axons. It promotes tubulin polymerization and stabilizes microtubules. The biological activity of tau is regulated by its degree of phosphorylation. 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.

Tau phosphorylation involves numerous kinases: glycogen synthase kinase 3 $\beta$  (GSK-3 $\beta$ ), 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. Combined tau protein kinase II (TPKII), which consists of Cdk5 and GSK-3 $\beta$ , is the most potent phosphorylation agent indirectly involved in the regulation of the phosphorylation state of tau in neuronal cells. 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. Normal tau has approximately eight phosphorylation sites. The abnormal phosphorylation occurs usually on serine and threonine residues. Specifically, TPKII phosphorylates Ser<sup>202, 404</sup>. GSK-3 $\beta$  transfection phosphorylates Ser<sup>199, 202, 235, 396, 404, 413</sup> and Thr<sup>205, 231</sup>. These sites are among the major abnormal phosphorylation sites of tau. Phosphorylation on these sites reduces the ability of a given tau species

to promote microtubule self-assembly. Okadaic acid increases phosphorylation at Thr<sup>231</sup> and Ser<sup>235, 396, 404</sup>. Phosphorylated Ser<sup>422</sup> was found in the biopsies of brains from patients with Down syndrome, amyotrophic lateral sclerosis, corticobasal degeneration, and Pick's disease. It was absent from a control group of normal brains. The opposite process, tau dephosphorylation, is controlled by various protein phosphatases expressed in neurons. Protein phosphatases PP2A and PP2B efficiently dephosphorylate tau *in vitro* and restore biological activity in the assembly of microtubules.

Recently it was discovered that propyl isomerase (Pin 1) interacts with tau hyperphosphorylated on Thr<sup>231</sup> and restores the ability of tau to bind to microtubules.

#### Reagent

The antibody is supplied as a solution in Dulbecco's phosphate buffered saline (without Mg<sup>2+</sup> and Ca<sup>2+</sup>), pH 7.3, with 50% glycerol, 1.0 mg/ml BSA (IgG and protease free) and 0.05% sodium azide.

#### Precautions and Disclaimer

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

#### Storage/Stability

Store at -20 °C. For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

#### Product Profile

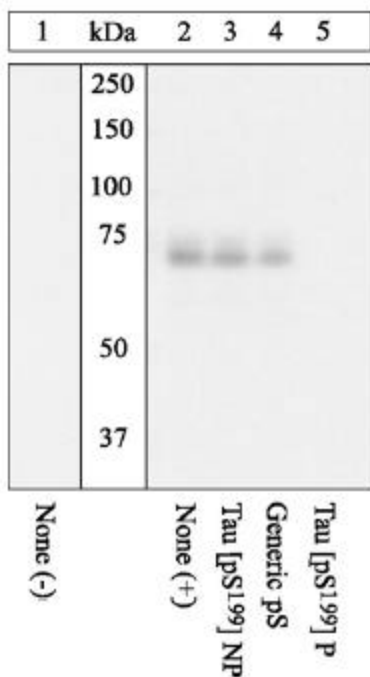
The supplied reagent is sufficient for 10 blots.

A recommended working dilution 1:1000 is determined by immunoblotting using recombinant human Tau treated with GSK-3 $\beta$ .

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

Peptide Competition

- Human recombinant tau added to background extracts and left untreated (1) or treated with GSK-3β (2-5) were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF.
- Membranes were blocked with a 5% BSA-TBST buffer for one hour at room temperature.
- 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 immunogen
- After preincubation membranes were incubated with tau (pSer<sup>199</sup>) antibody in a 1% BSA-TBST buffer for two hours at room temperature,
- After washing, membranes were incubated with goat F(ab')<sub>2</sub> anti-rabbit IgG-HRP and signals were detected using the Pierce SuperSignal™ method.



Data show the specificity of Anti-Phospho-Tau (pSer<sup>199</sup>) for the phosphorylated protein since no blocking is observed in the presence of the non-phosphorylated immunizing peptide.

**References**

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