

## Product Information

### Anti-phospho-Tau (pThr<sup>181</sup>)

Developed in Rabbit, Affinity Isolated Antibody

Catalog Number **T 8194**

#### Product Description

Anti-phospho-Tau (pThr<sup>181</sup>) is developed in rabbit using a synthetic phosphopeptide derived from the region of human tau that contains threonine 205 as immunogen. 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 (pThr<sup>181</sup>) recognizes human tau (pThr<sup>181</sup>) (45-68 kDa). It has been used in immunoblotting.

Tau is a microtubule-associated phosphoprotein (MAP), localized in neuronal axons. It promotes tubulin polymerization and stabilizes microtubules.<sup>1</sup> The biological activity of tau is regulated by its degree of phosphorylation.<sup>1,2</sup> 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.<sup>3,4</sup>

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.<sup>1,2,5,6,7</sup> 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.<sup>6,8</sup> 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.<sup>10</sup> 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-3 $\beta$  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.<sup>11</sup> Phosphorylation on these sites reduces the ability of a given tau species to promote microtubule self-assembly.<sup>11,12</sup> 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, amyotrophic lateral sclerosis, corticobasal degeneration, and Pick's disease. It was absent from control group of normal brains.<sup>13</sup>

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.<sup>3,10,14</sup>

Recently 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

The antibody is supplied at approximately 0.5 mg/ml in 100  $\mu$ l of Dulbecco's phosphate buffered saline (without Mg<sup>2+</sup> and Ca<sup>2+</sup>), pH 7.3, with 1.0 mg/mL BSA (IgG, protease free) and 0.05% sodium azide as a preservative.

#### 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 hazards and safe handling practices.

#### Storage/Stability

Store at  $-70^{\circ}\text{C}$ . 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

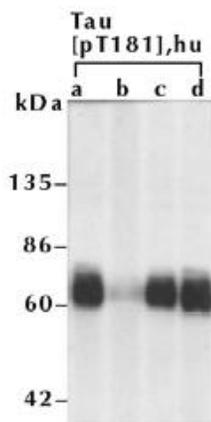
Sufficient for 10 blots.

A recommended working concentration of 0.1 to 1.0 µg/ml is determined by immunoblotting using cell extracts from African green monkey kidney (CV-1).

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

### Peptide Competition

1. Extracts from CV-1 cells stably expressing human four repeat tau and SV40 small T antigen to induce specific inhibition of PP2A were resolved by SDS-PAGE on a 4-20% polyacrylamide gel and transferred to nitrocellulose.
2. Membranes were blocked with a 4% BSA-TBST buffer overnight at 4 °C.
3. After blocking, membranes were preincubated with different peptides as follow:  
Lane a no peptide  
Lane b immunogen  
Lane c non phosphorylated peptide corresponding to the immunogen  
Lane d a generic phosphothreonine containing peptide
4. After preincubation, membranes were incubated with 1 µg/mL Tau (pThr<sup>181</sup>) antibody for two hours at room temperature in a 1% BSA-TBST buffer.
5. After washing, membranes were incubated with goat F(ab')<sub>2</sub> anti-rabbit IgG alkaline phosphatase and bands were detected using the Tropix WesternStar™ method.



The data show that only the peptide corresponding to Tau (pThr<sup>181</sup>) blocks the antibody signal, thereby demonstrating the specificity of the antibody.

### References

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