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Development and characterization of a novel monoclonal antibody to Tau p217 in Alzheimer's disease murine models and human disease tissue

INTRODUCTION

Phosphorylated tau have emerged as potential fluid-based biomarkers for Alzheimer's disease (AD) (1). Establishing a fluid-based biomarker, which measures analytes from patient cerebrospinal fluid (CSF) or plasma that associates with disease, would accelerate clinical research in treating this devastating neurodegenerative disease. Tau is phosphorylated at multiple sites. Amongst several specific tau phosphorylation sites, phosphorylation at threonine 217 (pTau217) has recently emerged as a potential reliable biomarker as levels of pTau217 increase in autosomal dominant AD patients (2) and may discriminate AD patients from non-AD patients compared to other tau phosphorylation sites (3,4). Measurement of fluid-based biomarkers largely leverage immuno-based technologies, which include enzyme-linked immunosorbent assay (ELISA) assays, immunoassays with electrochemiluminescence detection (ECL), single molecule arrays, and immunoprecipitation mass spectrometry (IP-MS). These assays require antibodies to the target protein of the highest specificity and sensitivity.

To improve on these assays, we sought to develop a rabbit monoclonal antibody to pTau217. Screening of rabbit monoclonal antibody libraries identified a clone, E9Y4S, that exhibited properties specific to pTau217. By western, we detected bands consistent with tau from WT mice that were absent in lambda phosphatase-treated tissue as well as tau KO brain lysates. The E9Y4S clone also specifically detected phosphorylated tau 217 peptide without reactivity to the corresponding non-phosphorylated tau. Using the E9Y4S clone, we developed a sandwich ELISA-compatible antibody pair and plate assay that detected pTau217 in rodent brain tissue. Moreover, we were able to detect elevated p217 levels in human AD brain tissue compared to non-diseased controls. Finally, we used the pTau217 ELISA to detect elevated levels of pTau217 in plasma from the TauP301S transgenic mouse model compared to WT controls, suggesting that our identified pTau217 ELISA pair and assay could be used to detect pTau217 in biofluids. Together, our data suggest the newly identified E9Y4S pTau217 monoclonal rabbit antibody is highly specific and sensitive to pTau217 in human and rodent AD tissue as well as rodent AD biofluids.

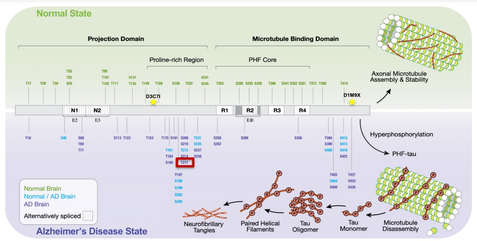

Diagram 1: Tau hyperphosphorylation in Alzheimer's disease
Tau, a microtubule-associated protein (MAP), exhibits altered and increased phosphorylation patterns via changes in tau kinase/phosphatase activity shifts. As a result, tau's capacity for microtubule stabilization is impaired, leading to increased microtubule catastrophe and faulty cargo trafficking. Importantly, pathological hyperphosphorylation of tau increases its susceptibility to aggregate into paired helical filaments, which form into large intracellular neurofibrillary tangles (NFTs), a noted hallmark of AD that is visible in diseased tissue. Microtubule destabilization and NFTs both contribute to the neurotoxicity and neurodegeneration associated with AD and tauopathies.

METHODS

Phospho-Tau (Thr217) (E9Y4S) Rabbit mAb was generated as a recombinant rabbit monoclonal antibody. Briefly, rabbits were injected with peptide antigens. After screening rabbit bleeds for immune responses, we collected the spleens of immune-responsive rabbits. We screened a library of 960 spleen clones for specificity and sensitivity to pTau217 by analyzing pTau peptides and tau-expressing tissue (+/- λ phosphatase) using ELISA/Dot Blots and Western analysis, respectively. We cloned and recombinantly expressed E9Y4S for further analysis and characterization. See CST Western Blot Protocols for more details.

ELISA was performed using PathScan® RP Phospho-Tau (Thr217) Sandwich ELISA Kit #59672. Analysis was performed on recombinant Tau protein and rodent brain lysates (+/- λ phosphatase). Additional analysis was performed on AD/non-diseased human brains. Analysis of plasma was performed using blood from control and TauP301S mice. See CST PathScan® RP Sandwich ELISA protocols for more details.

Affinity and binding kinetics were measured using the Octet RED96 System (Sartorius). Briefly, indicated peptides were immobilized streptavidin biosensors (SAX2). To measure association, peptide-immobilized sensors (indicated phospho- and non-phospho) were dipped into wells containing dilution series of relevant mAb for indicated times. Dissociation was measured by transferring the biosensors to PBS-containing wells for indicated times.

Key Antibody Products Used	Catalog #
Phospho-Tau (Thr217) (E9Y4S) Rabbit mAb	51625
Phospho-Tau (Thr217) Matched Antibody Pair	70933
PathScan® RP Phospho-Tau (Thr217) Sandwich ELISA Kit	59672
PathScan® RP Phospho-Tau (Thr217) Chemiluminescent Sandwich ELISA Kit	87749
Tau (D1M9X) XP® Rabbit mAb (Asp430)	46687
Tau (D3C7I) Rabbit mAb (Pro160)	79039*

***contact CST for availability**

Validation of phospho-Tau (Thr217) (E9Y4S) Rabbit mAb

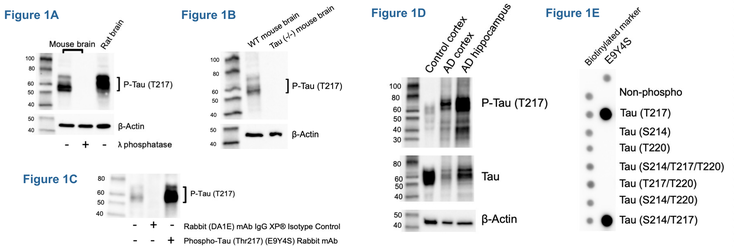

Figure 1: Monoclonal antibody validation of phospho-Tau (Thr217) (E9Y4S) Rabbit mAb by western blot, immunoprecipitation, and peptide dot blot
Phospho-Tau (Thr217) (E9Y4S) Rabbit mAb was validated by western blot or immunoprecipitation on various models. (A) E9Y4S was tested on rodent tissue with phospho-specificity determined by λ phosphatase-treated mouse brain lysate. (B) Target specificity to tau protein was determined by comparing WT mouse brain to a confirmed tau -/- brain. (C) Immunoprecipitation of Tau Thr217 was performed on mouse brain tissue extracts, showing enrichment compared to the input. (D) Enhanced pTau217 signal was detected in AD cortex/hippocampus compared to control cortex. (E) Specificity to T217 was evaluated by a peptide dot blot immunoassay utilizing a control and various phospho-peptides to T217 and surrounding sites, S214 and T220. E9Y4S detects T217 and T217/S214.

PathScan® RP Phospho-Tau (Thr217) Sandwich ELISA Kit detects pTau217 in rodent and human brain tissue samples

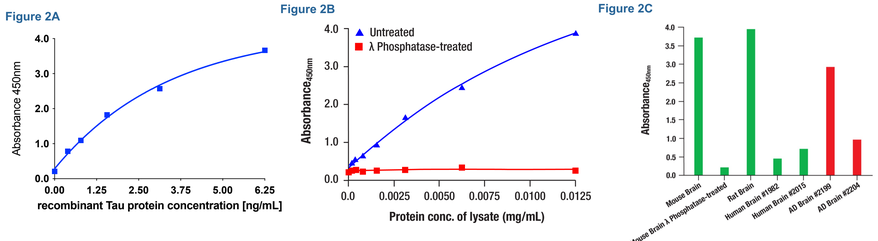

Figure 2: PathScan® RP Phospho-Tau (Thr217) Sandwich ELISA Kit detects pTau217 in rodent and human brain tissue samples
PathScan® RP Phospho-Tau (Thr217) Sandwich ELISA Kit detects phosphorylation of tau at Thr217 in rodent and human control/AD tissue. (A) Sensitivity of PathScan® RP Phospho-Tau (Thr217) Sandwich ELISA Kit was measured using recombinant Tau protein at indicated concentrations. (B) Sensitivity of PathScan® RP Phospho-Tau (Thr217) Sandwich ELISA Kit in brain tissue was measured using mouse brain tissue at indicated lysate protein titrations (+/- λ phosphatase). (C) Relative levels of pTau217 from rodent brain (+/- λ phosphatase) and human AD/Control brain were measured using PathScan® RP Phospho-Tau (Thr217) Sandwich ELISA Kit.

Binding Kinetics of Phospho-Tau (Thr217) (E9Y4S) Rabbit mAb and Competitor mAb

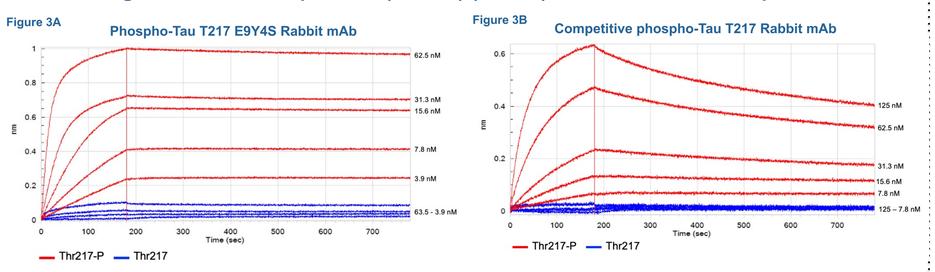

Figure 3C

Loading Sample ID	Vendor	KD (M)	k_{on}(1/Ms)	k_{dis}(1/s)	Full R^2
Thr217-P	Cell Signaling Technology Phospho-Tau T217 E9Y4S mAb	2.94E-11	3.51E+05	1.03E-05	0.9985
Thr217-P	Competitor Phospho-Tau T217 mAb	5.97E-09	1.15E+05	6.89E-04	0.9972

Figure 3: The binding kinetics of phospho-Tau (Thr217) (E9Y4S) Rabbit mAb and a commercially available competitor mAb were measured using bio-layer interferometry (BLI) on the Octet RED96 (Sartorius). Relevant biotinylated peptides were immobilized to High Precision Streptavidin 2.0 (SAX2, Sartorius) biosensors, which were then dipped into wells containing a 2-fold dilution series of either. (A) Phospho-Tau (Thr217) (E9Y4S) Rabbit mAb or (B) a commercially available competitor mAb. Sensorgrams show binding to the phosphorylated Thr217 peptide in red, and the non-phosphorylated Thr217 peptide in blue. Concentrations tested ranged from 250 nM to 3.9 nM. Global curve-fitting was performed using a bivalent analyte model; only the concentrations that passed that fitting are shown. (C) Association rates (k_{on}), dissociation rates (k_{dis}), dissociation constants (KD), as well as the coefficient of determination (Full R^2) are shown for each mAb .

Detection of pTau217 in Tg-Tau mouse plasma

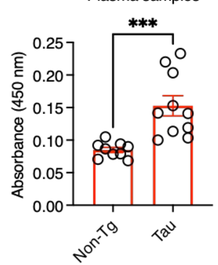

Figure 4: PathScan® RP Phospho-Tau (Thr217) Sandwich ELISA Kit detects pTau217 in plasma from Tau mouse models
The PathScan® Phospho-Tau (Thr217) Sandwich ELISA Kit #59672 discriminates phosphorylation of tau at Thr217 in plasma from 3-month-old Tau P301S mice (Tau) compared to control mice (Non-Tg). Data kindly provided by Dr. Ping-Chieh Pao and Dr. Li-Huei Tsai (MIT) and used with permission.

CONCLUSIONS

- CST has developed a highly sensitive and specific Phospho-Tau (Thr217) (E9Y4S) Rabbit mAb that can detect Tau phosphorylation at pTau217.
- Phospho-Tau (Thr217) (E9Y4S) Rabbit mAb can be used to detect pTau217 using Western blot analysis and ELISA to measure pTau217 in human/rodent tissue and plasma.
- Phospho-Tau (Thr217) (E9Y4S) Rabbit mAb binds to pTau217 with higher avidity and stronger binding kinetics than a commercially available competitor antibody against pTau217.

REFERENCES

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Acknowledgements

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