

Tau-441 (2N4R) P301S Mutant Monomers (CHO-expressed, N- glycosylated)



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Human Recombinant Tau-441 (2N4R) P301S
Mutant Monomers (CHO-expressed, N-
glycosylated)
Catalog No. SPR-515

distributed in the US/Canada by:

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Product Name

Tau-441 (2N4R) P301S Mutant Monomers (CHO-expressed, N-glycosylated)

Description

Human Recombinant Tau-441 (2N4R) P301S Mutant Monomers (CHO-expressed, N-glycosylated)

Applications

WB, SDS PAGE, In vitro Assay

Concentration

Lot/batch specific. See included datasheet.

Conjugates

N-term histidine tag & TEV site

Nature

Recombinant

Species

Human

Expression System

Chinese Hamster Ovary (CHO)

Amino Acid Sequence

GGSHHHHHHHHHHGGSGGSENLYFQGM AEP RQEF EVMEDHAGTYGLGDRKDQGGYTMHQDQEGD TDAGLKESPLQTPTE
DGSEEPGSETSDAKSTPTAEDVTAPLVDEGAPGKQAAAQPHTEIPEGTTAEAGIGDTPSLEDEAAGHV TQARMVSKSKDGTG
SDDKKAKGADGKTKIATPRGAAPPGQKGQANATRIPAKTPPAPKTPSSGEPKSGDRSGYSSPGSPGTPGSRSRTPSLPTPPT
REPKKVAVVRTPPKSPSSAKSRLQTAPVMPDLK N V K S K I G S T E N L K H Q P G G G K V Q I I N K K L D L S N V Q S K C G S K D N I K H V S G G G

SVQIVYKPVDSLKVTSCGSLGNIHHKPGGGQVEVKSEKLDKDRVQSKIGSLDNITHVPGGGNKKIETHKLTFRENAKAKTDH
GAEIVYKSPVVSAGDTSRHLNSVSSTGSIDMVDSPLATLADEVASLAKQGL

Purity

>95%

Protein Length

441 aa (excluding tag), 466 aa (including tag)

Protein Size

48.609 kDa

Field Of Use

Not for use in humans. Not for use in diagnostics or therapeutics. For in vitro research use only.

Properties

Storage Buffer

1X PB pH 7.4

Storage Temperature

-80°C

Shipping Temperature

Dry Ice. Shipping note: Product will be shipped separately from other products purchased in the same order.

Purification

Affinity Purified and Size Exclusion

Cite This Product

Human Recombinant Tau-441 (2N4R) P301S Mutant Monomers (CHO-expressed, N-glycosylated) (StressMarq Biosciences Inc., Victoria BC CANADA, Catalog # SPR-515)

Certificate Of Analysis

Protein certified >95% pure on SDS-PAGE & Nanodrop analysis

Other Relevant Information

CHO expression in mammalian cell line may lead to more "human" like phosphorylation/glycosylation patterns. For corresponding PFFs, see catalog# SPR-516.

Biological Description

Alternative Names

MAPT, intracellular neurofibrillary tangles, NFTs, paired helical filaments, PHFs, 2N4R

Research Areas

Alzheimer's Disease, Neurodegeneration, Neuroscience, Tangles & Tau


Swiss Prot

P10636-8

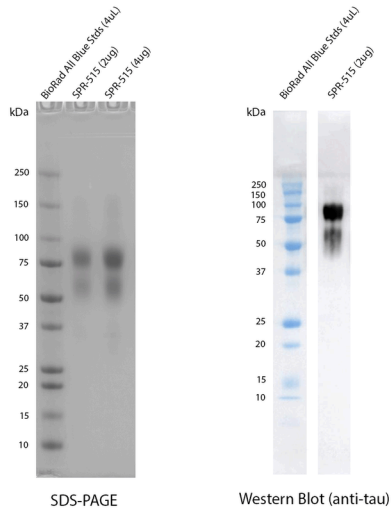
Scientific Background

Mammalian N-glycosylation is present on CHO-secreted tau 2N4R, which contributes to slower migration on SDS-PAGE than E.coli or Baculovirus/Sf9 expressed tau (1, 2). N-glycosylated tau has been identified in human AD-diseased brains, but not healthy brains, and may precede tau hyperphosphorylation (3, 4). N-glycosylation of Tau has been demonstrated to affect its aggregation propensity (5). The tau P301S mutation is associated with early onset neurodegeneration, and functionally reduces microtubule assembly and stimulates fibril assembly (6, 7). Our CHO-expressed Tau 2N4R P301S will readily form fibrils in the absence of heparin and contains mammalian post-translational modifications that may better mimic tau in human AD-brains.

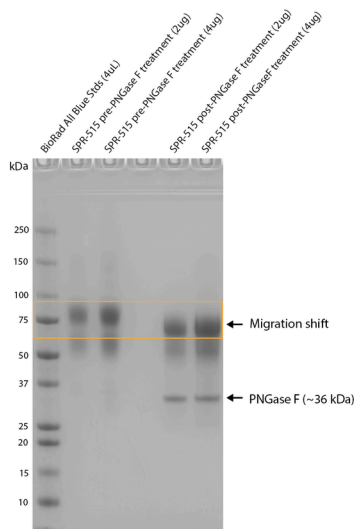
References

1. Guo et al., 2019. A pathogenic tau fragment compromises microtubules, disrupts insulin signaling and induces the unfolded protein response. *Acta Neuropathologica Communications*. DOI: 10.1186/s40478-018-0651-9 
 2. Losev et al., 2020. Differential effects of putative N-glycosylation sites in human Tau on Alzheimer's disease-related neurodegeneration. *Cellular and Molecular Life Sciences*. DOI: 10.1007/s00018-020-03643-3
 3. Zhang et al., 2020. Integrative glycoproteomics reveals protein N-glycosylation aberrations and glycoproteomic network alterations in Alzheimer's disease. *Sci. Adv.* DOI: 10.1126/sciadv.abc5802
 4. Liu et al., 2002. Role of glycosylation in hyperphosphorylation of tau in Alzheimer's disease. *FEBS*. DOI: 10.1016/S0014-5793(02)02228-7
 5. Losev et al., 2019. Novel model of secreted human tau protein reveals the impact of the abnormal N-glycosylation of tau on its aggregation propensity. *Sci. Rep.* <https://doi.org/10.1038/s41598-019-39218-x>
 6. Bugiani et al., 1999. Frontotemporal Dementia and Corticobasal Degeneration in a Family with a P301S Mutation in Tau. *J Neuropathol Exp Neurol*. doi: 10.1097/00005072-199906000-00011.
 7. Goedert and Crowther, 1999. Effects of frontotemporal dementia FTDP-17 mutations on heparin-induced assembly of tau filaments. *FEBS Lett*. DOI: 10.1016/s0014-5793(99)00508-6
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Product Images



SDS-PAGE and anti-tau western blot of SPR-515. The majority of CHO-expressed tau 2N4R P301S runs higher (75-100 kDa) than E.coli expressed tau (50-75 kDa) due to post-translational modifications as observed on a 5-12% gradient Bis-Tris gel (left). Tau was confirmed by running on a 12% Tris-Glycine gel, transferring to nitrocellulose, and blotting with 1:1000 anti-tau rabbit polyclonal antibody SPC-801 primary antibody, followed by 1:4000 goat anti-rabbit HRP (right). Exposure time 1 second after 5 minute incubation with chemiluminescent HRP substrate (Moss).



PNGase F treatment of SPR-515 shows an observable shift in apparent MW, indicating the presence of N-glycosylation. Monomers were treated with PNGase F (NEB), a glycosidase which specifically cleaves between the innermost GlcNAc and asparagine residues of N-linked oligosaccharides, and incubated at 37°C for 1 hour and run on a 5-12% gradient Bis-Tris gel.

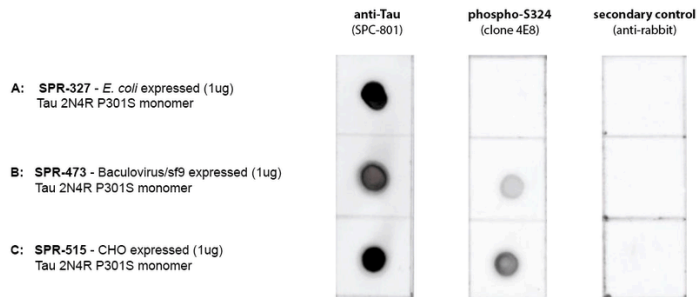
| Site | Example peptide (CHO-expressed tau 2N4R P301S) | N-glycosylation motif (N-X-S/T/C) present? | Modification | Best Ascore | Localization Probability | No PNGase F | | With PNGase F | |
|------|---|--|--------------|-------------|--------------------------|----------------------|----------------------|----------------------|----------------------|
| | | | | | | Modified/Total (CID) | Modified/Total (HCD) | Modified/Total (CID) | Modified/Total (HCD) |
| N167 | GQAnATRIPAK | Yes | Deamidated | 55.92 | 100% | 0/0 | 0/0 | 3/3 | 0/0 |
| N255 | LQTAPVPMPLK _n VK | No | Deamidated | 1,000.00 | 100% | 2/2 | 3/8 | 2/4 | 2/8 |
| N265 | IGSTEnLK | No | Deamidated | 1,000.00 | 100% | 15/40 | 14/54 | 16/74 | 13/82 |
| N279 | VQIInKK | No | Deamidated | 91.37 | 100% | 24/34 | 10/18 | 18/62 | 18/61 |
| N286 | LDLSnVQSK | No | Deamidated | 44.72 | 100% | 2/65 | 2/57 | 2/120 | 1/125 |
| N327 | CGSLGnIHHKPGGGQVEVK | No | Deamidated | 45.36 | 100% | 1/3 | 5/6 | 0/0 | 0/0 |
| N359 | IGSLDnITHVPGGGnKK | Yes | Deamidated | 1,000.00 | 100% | 15/37 | 17/37 | 65/94 | 63/97 |
| N368 | IGSLDNITHVPGGGnKK | No | Deamidated | 1,000.00 | 100% | 1/37 | 2/37 | 16/94 | 4/97 |
| N381 | LTFREnAK | No | Deamidated | 1,000.00 | 100% | 10/11 | 11/13 | 0/0 | 3/3 |
| N410 | SPVVS ^{GD} TS ^{PR} HL ^S n ^V S ST GS ^I DMVDSPQLATLADEV ^S AS ^L A | Yes | Deamidated | 17.46 | 99% | 0/1 | 0/0 | 0/1 | 1/2 |

Modified/Total deamidation spectrum counts as determined by mass spectrometry of SPR-515 before and after PNGase F treatment identifies potential N-glycosylation sites at N167, N359 and N410. Blue color indicates deamidation sites that match the N-glycosylation motif (N-X-S/T/C) and have a higher deamidation count after PNGase F treatment. No deamidation was present at N167 or N410 without PNGase F, suggesting these residues are protected from nonspecific deamidation by N-glycosylation. Some deamidation was present at N359 without PNGase F treatment, indicating a population of monomers is not glycosylated at this position. Several non-consensus, non-PNGase F-dependent deamidation sites were present, which may have occurred during production or the mass spectrometry workflow. Both CID and HCD fragmentation methods were used to improve sequence coverage and deamidation detection. Overall protein sequence coverage was 82%, with a localization probability cutoff set at $\geq 95\%$.

| Site | Modification | Best Ascore | Localization Probability | P301S tau 2N4R (CHO) CID | P301S tau 2N4R (CHO) HCD |
|------|--------------|-------------|--------------------------|--------------------------|--------------------------|
| T17 | Phospho | 17.01 | 100% | 0 / 456 | 1 / 513 |
| Y29 | Phospho | 40.91 | 100% | 0 / 365 | 8 / 296 |
| T30 | Phospho | 9.40 | 89% | 0 / 365 | 1 / 296 |
| S262 | Phospho | 33.98 | 100% | 6 / 40 | 5 / 54 |
| S324 | Phospho | 1,000.00 | 100% | 0 / 3 | 5 / 6 |
| T403 | Phospho | 7.65 | 85% | 0 / 343 | 2 / 191 |
| S404 | Phospho | 23.10 | 99% | 5 / 343 | 0 / 191 |

Modified/Total phosphorylation PTM spectrum counts reveal up to 7 phosphorylation sites on human P301S Tau 2N4R monomers expressed using CHO as determined by mass spectrometry. Both CID and HCD fragmentation methods were used to improve sequence coverage and deamidation detection. Protein

sequence coverage was 82%. Localization probability cutoff set at $\geq 80\%$ (yellow) or $\geq 95\%$ (green). Note: number of phosphorylation sites appear less than Baculovirus/Sf9 expressed tau 2N4R (see StressMarq cat# SPR-471, 472, 496 and 498).



Dot Blot of purified hTau (2N4R) P301S monomers (SPR-515) using Stressmarq's SPC-801 and a phospho-S324 Tau antibody (GeneBio Systems) comparing phosphorylation in E.coli-expressed, baculovirus/sf9-expressed, and CHO-expressed material. Protein was blotted on nitrocellulose, incubated with 1:1000 primary antibodies and/or 1:4000 secondary antibodies. Secondary control is goat-anti rabbit:HRP. Exposed 1 second.

Product Citations

Reviews

There are no reviews yet.