Anti-Phospho-Tau (S396) Antibody [SN62-09] ET1611-68

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Mouse, Rat, Human
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IHC-Fr
Molecular Wt:	Predicted band size: 79 kDa
Clone number:	SN62-09

Description: Tau, also known as MAPT (microtubule-associated protein tau), MAPTL, MTBT1 or TAU, is a 758 amino acid protein that localizes to the cytoplasm, as well as to the cytoskeleton and the cell membrane, and contains four Tau/MAP repeats. Expressed in neuronal tissue and existing as multiple alternatively spliced isoforms, Tau functions to promote microtubule assembly and stability and is thought to be involved in the maintenance of neuronal polarity. Tau may also link microtubules with neural plasma membrane components and, in addition to its role in microtubule stability, is also necessary for cytoskeletal plasticity. Tau is highly subject to a variety of post-translational modifications, including phosphorylation on serine and threonine residues, polyubiquitination (and subsequent proteasomal degradation) and glycation of specific Tau isoforms. Defects in the gene encoding Tau are associated with Alzheimers disease, pallido-ponto-nigral degeneration (PPND), corticobasal degeneration (CBD) and progressive supranuclear palsy (PSP).

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Ser396 of human Tau.

Positive control: SHSY5Y cell lysates, N2A, PC-12, rat brain tissue, mouse brain tissue, mouse kidney tissue.

Subcellular location: Cell membrane, Cell projection, Cytoplasm, Cytoskeleton, Membrane, Microtubule, Secreted.

Database links: SwissProt: P10636-8 Human | P10637 Mouse | P19332 Rat

Recommended Dilutions:

WB	1:500-1:2,000
IF-Cell	1:100-1:500
IF-Tissue	1:100-1:500
IHC-P	1:50-1:200
IHC-Fr	1:100

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

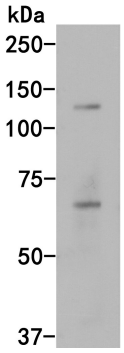

Fig1: Western blot analysis of Phospho-Tau (S396) on SHSY5Y cell lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ET1611-68, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:5,000 dilution was used for 1 hour at room temperature.

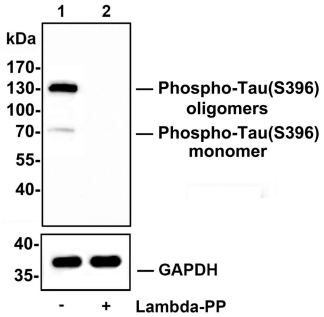

Fig2: Western blot analysis of Phospho-Tau(S396) on SHSY5Y cell lysates.

Lane 1: SHSY5Y cells, whole cell lysate, 10ug/lane
Lane 2: SHSY5Y cells treated with 2.8ug/ul lambda-PP for 30 minutes, whole cell lysates, 10ug/lane

All lanes :
Anti-Phospho-Tau(S396) antibody (ET1611-68) at 1/500 dilution. Anti-GAPDH antibody (ET1601-4) at 1/10,000 dilution. Goat Anti-Rabbit IgG H&L (HRP) (HA1001) at 1/200,000 dilution.

Predicted band size: 79 kDa
Observed band size: 70/130 kDa

Blocking and diluting buffer: 5% BSA.

Exposure time: 2 minutes 34 seconds

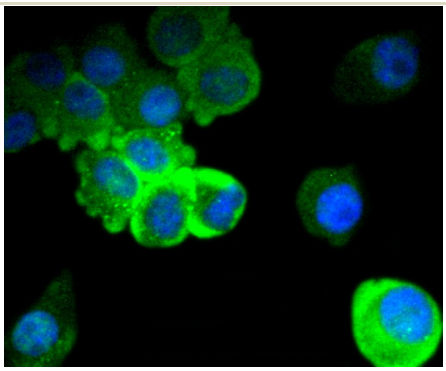

Fig3: ICC staining of Phospho-Tau (S396) in N2A cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1611-68, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

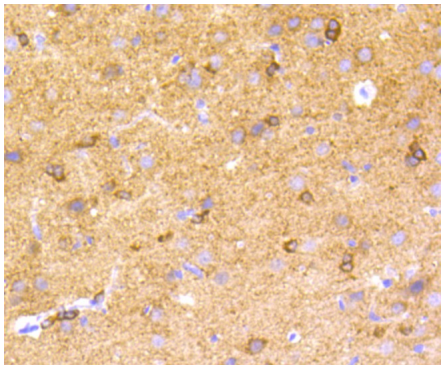

Fig4: Immunohistochemical analysis of paraffin-embedded rat brain tissue using anti-Phospho-Tau (S396) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1611-68, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

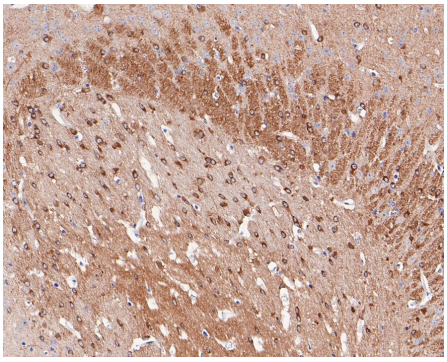

Fig5: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-Phospho-Tau (S396) antibody (ET1611-68) at 1/50 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1611-68) at 1/50 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

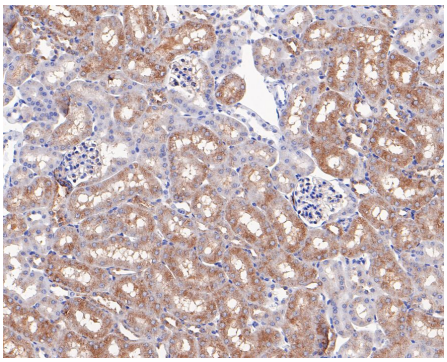

Fig6: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-Phospho-Tau (S396) antibody (ET1611-68) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1611-68) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

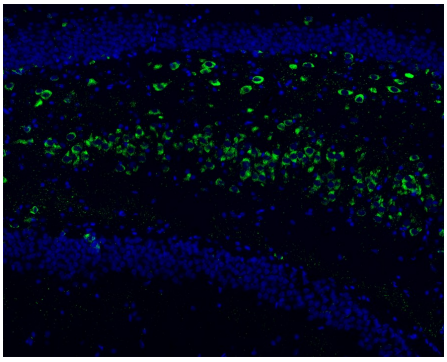

Fig7: Immunofluorescence analysis of frozen mouse hippocampus tissue labeling Phospho-Tau (S396) with Rabbit anti-Phospho-Tau (S396) antibody (ET1611-68).
The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with the primary antibody (ET1611-68, green) at 1/100 dilution overnight at 4℃, washed with PBS. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) was used as the secondary antibody at 1/200 dilution. Nuclei were counterstained with DAPI (blue). Image acquisition was performed with KFBIO KF-FL-400 Scanner.

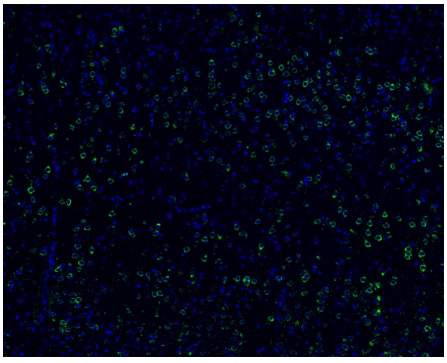

Fig8: Immunofluorescence analysis of frozen mouse cerebral cortex tissue labeling Phospho-Tau (S396) with Rabbit anti-Phospho-Tau (S396) antibody (ET1611-68).
The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with the primary antibody (ET1611-68, green) at 1/100 dilution overnight at 4℃, washed with PBS. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) was used as the secondary antibody at 1/200 dilution. Nuclei were counterstained with DAPI (blue). Image acquisition was performed with KFBIO KF-FL-400 Scanner.

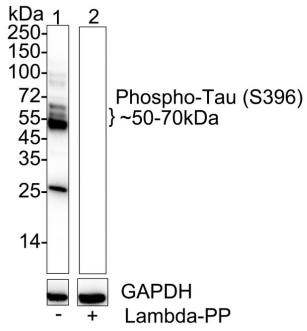

Fig9: Western blot analysis of Phospho-Tau (S396) on different lysates with Rabbit anti-Phospho-Tau (S396) antibody (ET1611-68) at 1/2,000 dilution.

Lane 1: Mouse brain tissue lysate
Lane 2: Mouse brain tissue lysate treated with lambda-PP for 30 minutes

Lysates/proteins at 40 µg/Lane.

Predicted band size: 79 kDa
Observed band size: 50-70, 130 kDa

Exposure time: 10 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1611-68) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature."

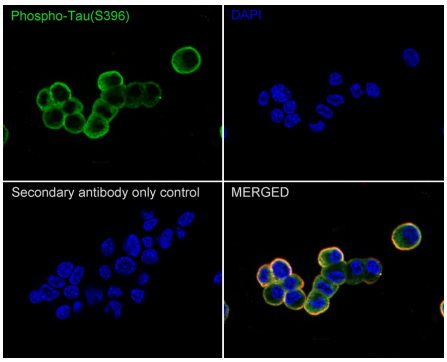

Fig10: Immunocytochemistry analysis of PC-12 cells labeling Phospho-Tau (S396) with Rabbit anti-Phospho-Tau (S396) antibody (ET1611-68) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Phospho-Tau (S396) antibody (ET1611-68) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Background References

1. Manczak M & Reddy PH Abnormal interaction of oligomeric amyloid- with phosphorylated tau: implications to synaptic dysfunction and neuronal damage. J Alzheimers Dis 36:285-95 (2013).
2. Murakami K et al. SOD1 (copper/zinc superoxide dismutase) deficiency drives amyloid protein oligomerization and memory loss in mouse model of Alzheimer disease. J Biol Chem 286:44557-68 (2011).