Phospho-Tau (Ser214/T217) Signaling Antibody Sampler Kit

HAK21128

Contains Product	Quantity	Applications	Species reactivity	MW(kDa)
p38 alpha / MAPK14 [ET1702-65]	20µl	WB,IF-Cell,IF-Tissue,IHC-P,FC	H,M,R	41 kDa
Phospho-p38 alpha (T180 + Y182) [HA722669]	20µl	WB,IF-Cell,FC	H,M,R	41 kDa
JNK1+JNK2+JNK3 [ET1601-28]	20µl	WB,IF-Cell,IF-Tissue,IHC-P,IP	H,M,R	48/53 kDa
Phospho-JNK1/2/3 (T183 + T183 + T221) [ET1609-42]	20µl	WB,IF-Cell,IF-Tissue,IHC-P,IP,FC,IHC-Fr	H,M,R	48/53 kDa
Tau [ET1612-44]	20µl	WB,IF-Cell,IF-Tissue,IHC-P,FC,IHC-Fr	H,M,R	79 kDa
Phospho-Tau (S214) [HA721797]	20µl	WB	H	79 kDa
Phospho-Tau (T217) [HA723091]	20µl	WB,IF-Cell,IHC-P,IHC-Fr	H,M,R	79 kDa
p70 S6 Kinase [HA722520]	20µl	WB,IHC-P,IP	H,M,R	59 kDa
Phospho-p70 S6 Kinase (T389) [HA721803]	20µl	WB,IF-Cell,IHC-P	H,M,R	59 kDa
HRP-Goat Anti-Rabbit IgG (H+L) [HA1001]	100µl	WB,ELISA,IHC-P	Rab	

Description: The Phospho-Tau (Ser214/T217) Signaling Antibody Sampler Kit provides an economical means of detecting the signaling cascade leading to tau phosphorylation at Ser214 and Thr217, using phospho-specific and control antibodies. The kit includes enough antibodies to perform two western blot experiments with each primary antibody.

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℃. Avoid repeated freeze / thaw cycles.

Background

Tau is a heterogeneous microtubule-associated protein that promotes and stabilizes microtubule assembly, especially in axons. Phosphorylation decreases the ability of tau to bind to microtubules. Neurofibrillary tangles are a major hallmark of Alzheimer's disease (AD); these tangles are bundles of paired helical filaments (PHFs) composed of hyperphosphorylated tau.

Numerous kinases, including p38 MAPK, JNK, CDK5, GSK3β, and SGK1, have been shown to phosphorylate tau at Ser214, which is found in AD and dementia with Lewy bodies. Another kinase that phosphorylates the Ser214 site on tau is the stress-activated protein kinase/Jun-amino-terminal kinase (SAPK/JNK). Tau phosphorylation at Thr217 has been identified as a highly specific biomarker of AD. One kinase identified to phosphorylate the Thr217 site on tau is p70 S6 kinase (S6K1). Phosphorylation of Thr389 most closely correlates with S6K1 activity in vivo.

Database links: UniProt ID: Q16539, P47811, P70618, Q16539, P47811, P70618, P45983, P45984, P53779, Q61831, Q91Y86, Q9WTU6, P49185, P49186, P49187, P45983, P45984, P53779, Q61831, Q91Y86, Q9WTU6, P49185, P49186, P49187, P10636, P10637, P19332, P10636-8 , P10637, P19332, P10636-8, P10637, P23443, Q8BSK8, P67999, P23443, Q8BSK8, 83840

Images

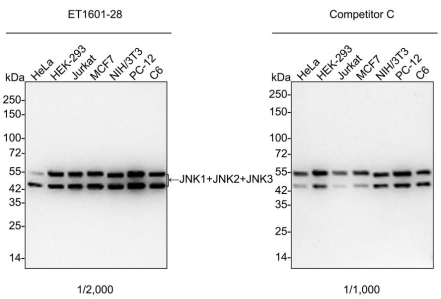

Fig1: Western blot analysis of JNK1+JNK2+JNK3 on different lysates with Rabbit anti-JNK1+JNK2+JNK3 antibody (ET1601-28) at 1/2,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HEK-293 cell lysate
Lane 3: Jurkat cell lysate
Lane 4: MCF7 cell lysate
Lane 5: NIH/3T3 cell lysate
Lane 6: PC-12 cell lysate
Lane 7: C6 cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 48/53 kDa
Observed band size: 48/53 kDa

Exposure time: 35 seconds; ECL: K1801;

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 (ET1601-28) at 1/2,000 dilution and competitor's antibody at 1/1,000 dilution were 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.

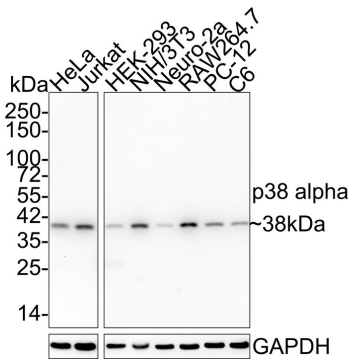

Fig2: Western blot analysis of p38 alpha on different lysates with Rabbit anti-p38 alpha antibody (ET1702-65) at 1/5,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: Jurkat cell lysate
Lane 3: HEK-293 cell lysate
Lane 4: NIH/3T3 cell lysate
Lane 5: Neuro-2a cell lysate
Lane 6: RAW264.7 cell lysate
Lane 7: PC-12 cell lysate
Lane 8: C6 cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 41 kDa
Observed band size: 38 kDa

Exposure time: 3 minutes;

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 (ET1702-65) at 1/5,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

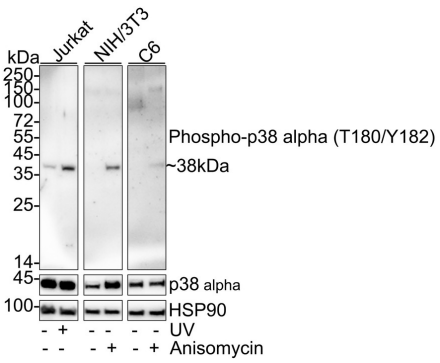

Fig3: Western blot analysis of Phospho-p38 alpha (T180 + Y182) on different lysates with Rabbit anti-Phospho-p38 alpha (T180 + Y182) antibody (HA722669) at 1/2,000 dilution and p38 alpha antibody (ET1702-65) at 1/1,000 dilution.

Lane 1: Jurkat cell lysate (20 µg/Lane)
Lane 2: Jurkat treated with UV for 1 hour cell lysate (20 µg/Lane)
Lane 3: NIH/3T3 cell lysate (20 µg/Lane)
Lane 4: NIH/3T3 treated with 25µg/mL Anisomycin for 30 minutes cell lysate (20 µg/Lane)
Lane 5: C6 cell lysate (20 µg/Lane)
Lane 6: C6 treated with 25µg/mL Anisomycin for 30 minutes cell lysate (20 µg/Lane)

Predicted band size: 41 kDa
Observed band size: 38 kDa
Exposure time: 3 minutes; ECL: K1802;
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 (HA722669) at 1/2,000 dilution and p38 alpha antibody (ET1702-65) at 1/1,000 dilution were 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.

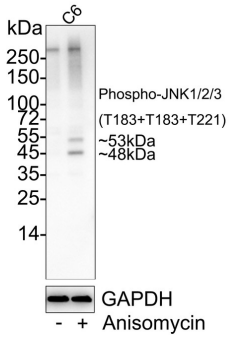

Fig4: Western blot analysis of Phospho-JNK1/2/3 (T183 + T183 + T221) on different lysates with Rabbit anti-Phospho-JNK1/2/3 (T183 + T183 + T221) antibody (ET1609-42) at 1/2,000 dilution.

Lane 1: C6 cell lysate
Lane 2: C6 treated with 25ug/mL Anisomycin for 30 minutes whole cell lysate.

Lysates/proteins at 20 µg/Lane.
Predicted band size: 48/53 kDa
Observed band size: 48/53 kDa
Exposure time: 2 minutes 6 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 (ET1609-42) 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.

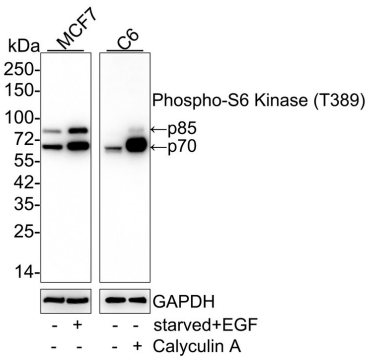

Fig5: Western blot analysis of Phospho-p70 S6 Kinase (T389) on different lysates with Rabbit anti-Phospho-p70 S6 Kinase (T389) antibody (HA721803) at 1/1,000 dilution.

Lane 1: MCF7 cell lysate
Lane 2: MCF7 starved for 4 hours then treated with 200ng/mL EGF for 15 minutes cell lysate
Lane 3: C6 cell lysate
Lane 4: C6 treated with 100nM Calyculin A for 30 minutes cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 59 kDa
Observed band size: 70/85 kDa

Exposure time: 46 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 (HA721803) at 1/1,000 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.

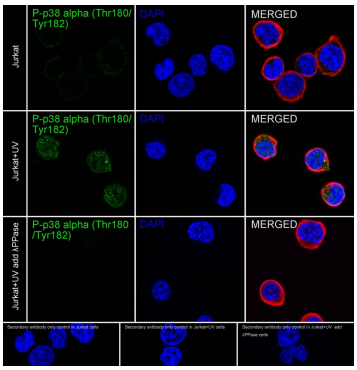

Fig6: Immunocytochemistry analysis of Jurkat cells treated with UV for 1 hour labeling Phospho-p38 alpha (T180 + Y182) with Rabbit anti-Phospho-p38 alpha (T180 + Y182) antibody (HA722669) at 1/50 dilution.

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-p38 alpha (T180 + Y182) antibody (HA722669) at 1/50 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.

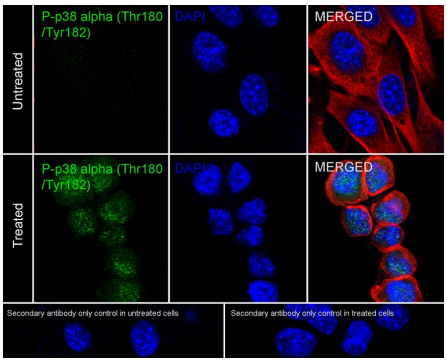

Fig7: Immunocytochemistry analysis of NIH/3T3 cells treated with 25µg/mL Anisomycin for 30 minutes labeling Phospho-p38 alpha (T180 + Y182) with Rabbit anti-Phospho-p38 alpha (T180 + Y182) antibody (HA722669) at 1/100 dilution.

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-p38 alpha (T180 + Y182) antibody (HA722669) 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.

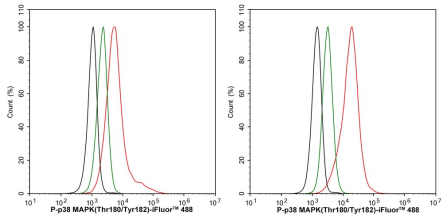

Fig8: Flow cytometric analysis of Jurkat cells untreated (left) or treated (right) with UV for 1 hour labeling Phospho-p38 alpha (T180 + Y182).

Cells were fixed and permeabilized. Then stained with the primary antibody (HA722669, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

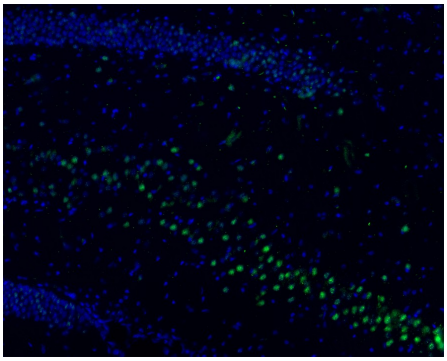

Fig9: Immunofluorescence analysis of frozen mouse hippocampus tissue labeling Phospho-JNK1/2/3 (T183 + T183 + T221) with Rabbit anti-Phospho-JNK1/2/3 (T183 + T183 + T221) antibody (ET1609-42).

The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with the primary antibody (ET1609-42, 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.

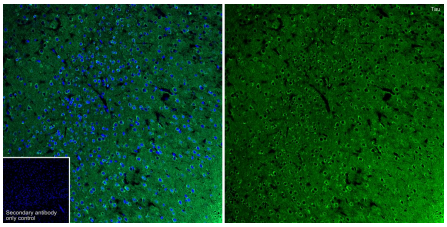

Fig10: Immunofluorescence analysis of frozen mouse brain tissue with Rabbit anti-Tau antibody (ET1612-44) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1612-44, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

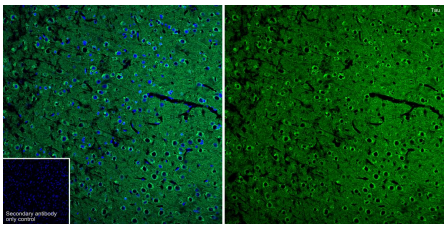

Fig11: Immunofluorescence analysis of frozen rat brain tissue with Rabbit anti-Tau antibody (ET1612-44) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1612-44, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

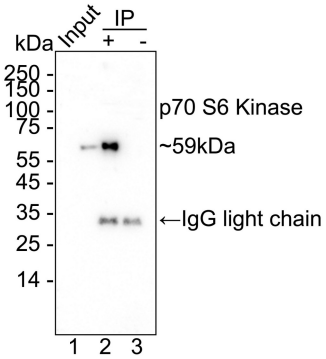

Fig12: p70 S6 Kinase was immunoprecipitated from 0.2 mg C6 cell lysate with HA722520 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA722520 at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: C6 cell lysate (input)
Lane 2: HA722520 IP in C6 cell lysate
Lane 3: Rabbit IgG instead of HA722520 in C6 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 20 seconds; ECL: K1801

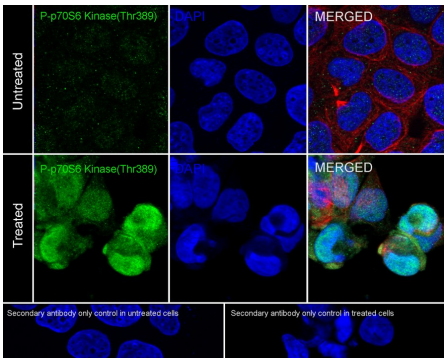

Fig13: Immunocytochemistry analysis of MCF7 cells treated with or without 20% FBS overnight then add 100nM Calyculin A for 30 minutes labeling Phospho-p70 S6 Kinase (T389) with Rabbit anti-Phospho-p70 S6 Kinase (T389) antibody (HA721803) at 1/100 dilution.

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-p70 S6 Kinase (T389) antibody (HA721803) 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. Janelidze S, Stomrud E, Smith R, Palmqvist S, Mattsson N, Airey DC, Proctor NK, Chai X, Shcherbinin S, Sims JR, Triana-Baltzer G, Theunis C, Slemmon R, Mercken M, Kolb H, Dage JL, Hansson O. Cerebrospinal fluid p-tau217 performs better than p-tau181 as a biomarker of Alzheimer's disease. Nat Commun. 2020 Apr 3;11(1):1683.
2. Barthélemy NR, Bateman RJ, Hirtz C, Marin P, Becher F, Sato C, Gabelle A, Lehmann S. Cerebrospinal fluid phospho-tau T217 outperforms T181 as a biomarker for the differential diagnosis of Alzheimer's disease and PET amyloid-positive patient identification. Alzheimers Res Ther. 2020 Mar 17;12(1):26.