Anti-Phospho-JNK1/2/3 (T183 + T183 + T221) Antibody [ST500]

ET1609-42

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP, FC, IHC-Fr
Molecular Wt:	Predicted band size: 48/53 kDa
Clone number:	ST500

Description: JNKs (c-Jun N-terminal kinases) belong to a family of MAP kinases that are involved in a variety of cellular processes, including transcriptional regulation and cellular proliferation, differentiation and development. JNK2 (c-Jun N-terminal kinase 2) and JNK3 (c-Jun N-terminal kinase 3) are 424 and 464 amino acid proteins, respectively, that each contain one protein kinase domain and use magnesium as a cofactor to catalyze the phosphorylation of target proteins, thereby playing a role in a variety of events throughout the cell. Both JNK2 and JNK3 exist as multiple alternatively spliced isoforms and are subject to post-translational phosphorylation on Thr 183 and Thr 221, respectively, an event which activates JNK2/JNK3 enzymatic activity. Defects in the gene encoding JNK3 are a cause of epileptic encephalopathy of the Lennox-Gastaut type, a group of epileptic disorders characterized by severe psychomotor delay and seizures.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Thr183 + Thr183 + Thr221 of Human JNK1 / 2 / 3 aa 161-204 / 427.

Positive control: NIH/3T3 cell lysate treated with Anisomycin, Hela cell lysate treated with Anisomycin, A431 cell lysate treated with UV40, 293 cell lysate treated with UV40, Hela cell lysate treated with UV40, Hela, NIH/3T3, HUVEC, human colon tissue, human endometrium tissue, mouse heart tissue.

Subcellular location: Cytoplasm, Membrane, Mitochondrion, Nucleus.

Database links: SwissProt: P45983 Human | P45984 Human | P53779 Human | Q61831 Mouse | Q91Y86 Mouse | Q9WTU6 Mouse | P49185 Rat | P49186 Rat | P49187 Rat

Recommended Dilutions:

WB	1:1,000-1:2,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:50-1:1,000
FC	1:50-1:100
IP	Use at an assay dependent concentration.
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

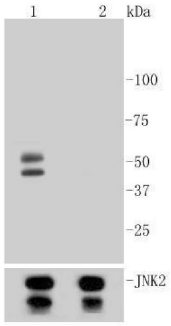

Fig1: Western blot analysis of Phospho-JNK1/2/3 (T183 + T183 + T221) on different lysates using anti-Phospho-JNK1/2/3 (T183 + T183 + T221) antibody at 1/500 dilution.
Lane 1: NIH/3T3 cell lysate, treated with Anisomycin
Lane 2: NIH/3T3 cell lysate, untreated

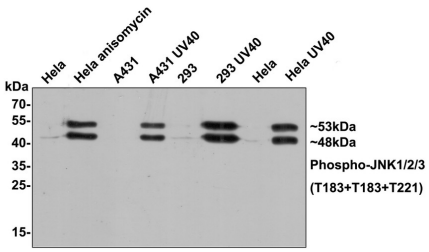

Fig2: Western blot analysis of Phospho-JNK1/2/3 (T183 + T183 + T221) on different lysates with Rabbit anti-Phospho-JNK1/2/3 (T183 + T183 + T221) antibody (ET1609-42) at 1/1,000 dilution.

Lane 1: Hela cell lysate, untreated
Lane 2: Hela cell lysate, treated with Anisomycin
Lane 3: A431 cell lysate, untreated
Lane 4: A431 cell lysate, treated with UV40
Lane 5: 293 cell lysate, untreated
Lane 6: 293 cell lysate, treated with UV40
Lane 7: Hela cell lysate, untreated
Lane 8: Hela cell lysate, treated with UV40

Lysates/proteins at 10 µg/Lane.

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

Exposure time: 2 minutes;

12% 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 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

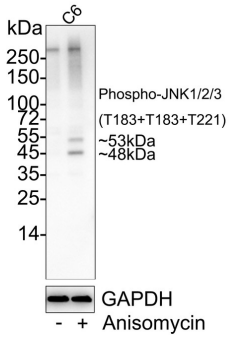

Fig3: Western blot analysis of Phospho-JNK1/2/3 (T183 + T183 + T221) on different lysates with Rabbit anti-Phospho-JNK1/2/3 (T183 + T183 + T221) antibody (ET1609-42) at 1/2,000 dilution.

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."

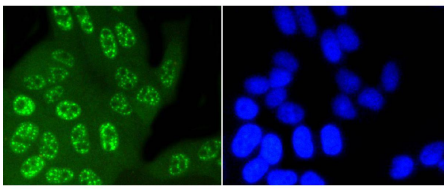

Fig4: ICC staining of Phospho-JNK1/2/3 (T183 + T183 + T221) in Hela cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1609-42, 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).

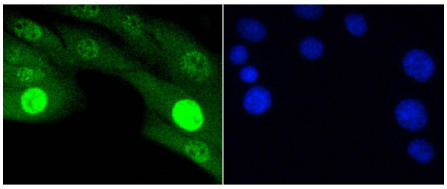

Fig5: ICC staining of Phospho-JNK1/2/3 (T183 + T183 + T221) in NIH/3T3 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1609-42, 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).

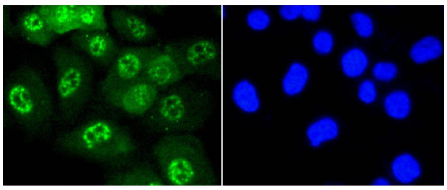

Fig6: ICC staining of Phospho-JNK1/2/3 (T183 + T183 + T221) in HUVEC cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1609-42, 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).

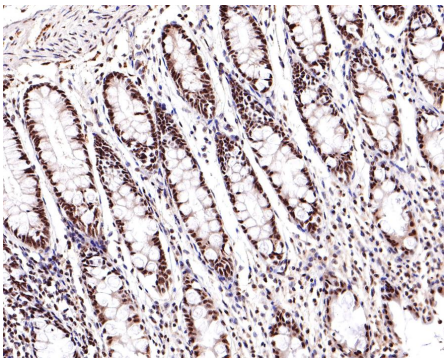

Fig7: Immunohistochemical analysis of paraffin-embedded human colon tissue with Rabbit anti-Phospho-JNK1/2/3 (T183 + T183 + T221) antibody (ET1609-42) at 1/400 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (ET1609-42) at 1/400 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.

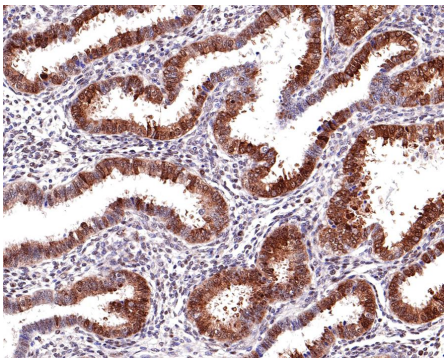

Fig8: Immunohistochemical analysis of paraffin-embedded human endometrium tissue with Rabbit anti-Phospho-JNK1/2/3 (T183 + T183 + T221) antibody (ET1609-42) at 1/400 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (ET1609-42) at 1/400 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.

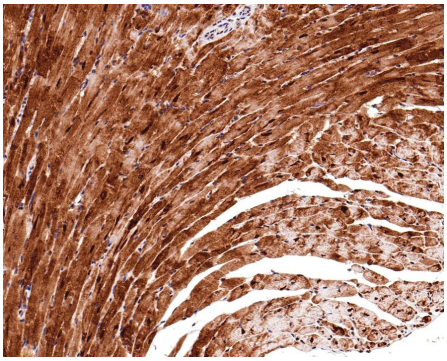

Fig9: Immunohistochemical analysis of paraffin-embedded mouse heart tissue with Rabbit anti-Phospho-JNK1/2/3 (T183 + T183 + T221) antibody (ET1609-42) at 1/400 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (ET1609-42) at 1/400 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.

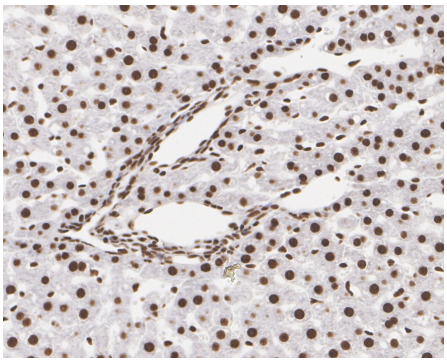

Fig10: Immunohistochemical analysis of paraffin-embedded rat liver tissue with Rabbit anti-Phospho-JNK1/2/3 (T183 + T183 + T221) antibody (ET1609-42) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (ET1609-42) at 1/1,000 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.

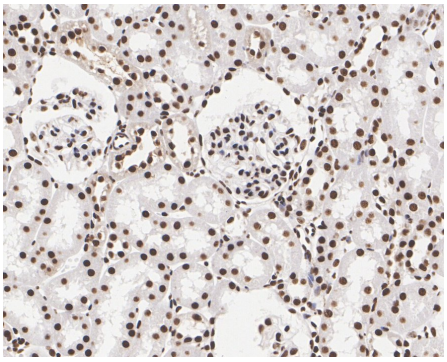

Fig11: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-Phospho-JNK1/2/3 (T183 + T183 + T221) antibody (ET1609-42) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (ET1609-42) at 1/1,000 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.

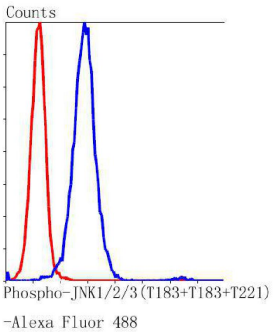

Fig12: Flow cytometric analysis of Phospho-JNK1/2/3 (T183 + T183 + T221) was done on Hela cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1609-42, 1/100) (blue). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; red).

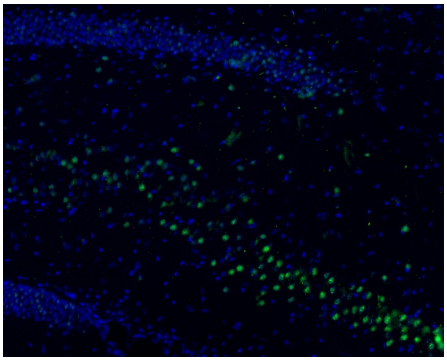

Fig13: Immunofluorescence analysis of frozen mouse hippocampus tissue labeling Phospho-JNK1/2/3 (T183 + T183 + T221) with Rabbit anti-Phospho-JNK1/2/3 (T183 + T183 + T221) antibody (ET1609-42).
The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with 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.

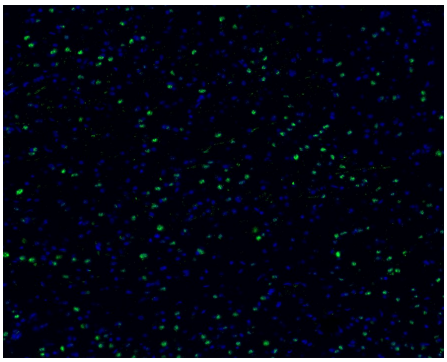

Fig14: Immunofluorescence analysis of frozen mouse cerebral cortex tissue labeling Phospho-JNK1/2/3 (T183 + T183 + T221) with Rabbit anti-Phospho-JNK1/2/3 (T183 + T183 + T221) antibody (ET1609-42).
The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed 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.

Background References

1. Kang K et al. Carnosic acid slows photoreceptor degeneration in the Pde6b(rd10) mouse model of retinitis pigmentosa. Sci Rep 6:22632 (2016).
2. Li C et al. Inhibitory effects of kaempferol on the invasion of human breast carcinoma cells by downregulating the expression and activity of matrix metalloproteinase-9. Biochem Cell Biol 93:16-27 (2015).