Anti-Phospho-JunD (S255) Antibody [JJ08-21] ET1701-35

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P
Molecular Wt:	Predicted band size: 35 kDa
Clone number:	JJ08-21

Description: Transcription factor JunD is a protein that in humans is encoded by the JUND gene. The protein encoded by this intronless gene is a member of the JUN family, and a functional component of the AP1 transcription factor complex. It has been proposed to protect cells from p53-dependent senescence and apoptosis. Alternate translation initiation site usage results in the production of different isoforms. The dominant negative mutant variant of JunD, known as ΔJunD or Delta JunD, is a potent antagonist of the ΔFosB transcript, as well as other forms of AP-1-mediated transcriptional activity. In the nucleus accumbens, ΔJunD directly opposes many of the neurological changes that occur in addiction (i.e., those induced by ΔFosB). ΔFosB inhibitors (drugs that oppose its action) may be an effective treatment for addiction and addictive disorders. Being an unnatural genetic variant, deltaJunD has not been observed in humans. JunD has been shown to interact with ATF3, MEN1, DNA damage-inducible transcript 3 and BRCA1.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Ser255 of Human JunD aa 251-270 / 347.

Positive control: HeLa cell lysate, K-562 cell lysate, NIH/3T3 cell lysate, PC-12 cell lysate, HepG2, HeLa, human small intestine tissue, mouse small intestine tissue, rat small intestine tissue, human breast tissue.

Subcellular location: Nucleus.

Database links: SwissProt: P17535 Human | P15066 Mouse | P52909 Rat

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:50-1:200

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

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

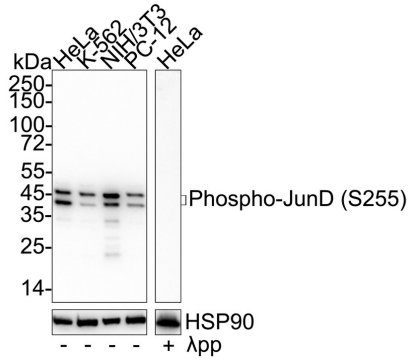

Fig1: Western blot analysis of Phospho-JunD (S255) on different lysates with Rabbit anti-Phospho-JunD (S255) antibody (ET1701-35) at 1/5,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: K-562 cell lysate
Lane 3: NIH/3T3 cell lysate
Lane 4: PC-12 cell lysate
Lane 5: HeLa cell lysate, the membrane treated with λpp for 1 hour

Lysates/proteins at 20 µg/Lane.

Predicted band size: 35 kDa
Observed band size: 40/45 kDa

Exposure time: 30 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 (ET1701-35) at 1/5,000 dilution was used in primary antibody dilution (K1803) at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

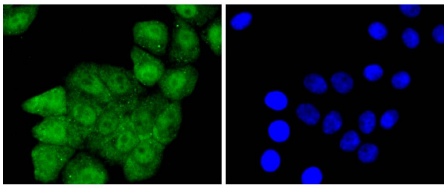

Fig2: ICC staining of Phospho-JunD (S255) in HepG2 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1701-35, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

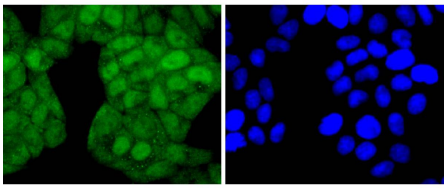

Fig3: ICC staining of Phospho-JunD (S255) in Hela cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1701-35, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

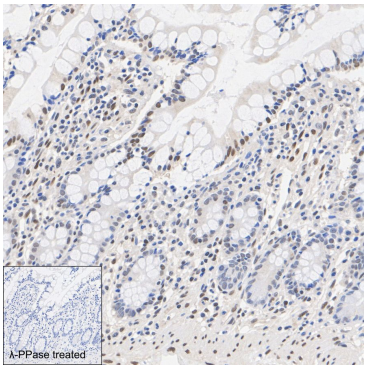

Fig4: Immunohistochemical analysis of paraffin-embedded human small intestine tissue untreated / treated with λpp with Rabbit anti-Phospho-JunD (S255) antibody (ET1701-35) at 1/200 dilution.

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 (ET1701-35) 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.

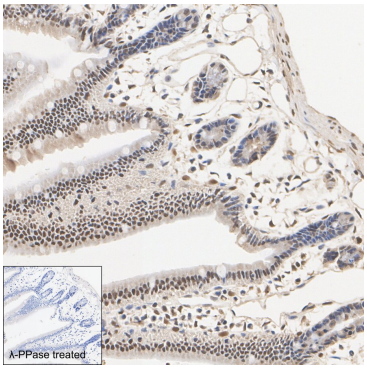

Fig5: Immunohistochemical analysis of paraffin-embedded mouse small intestine tissue untreated / treated with λpp with Rabbit anti-Phospho-JunD (S255) antibody (ET1701-35) at 1/200 dilution.

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 (ET1701-35) 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.

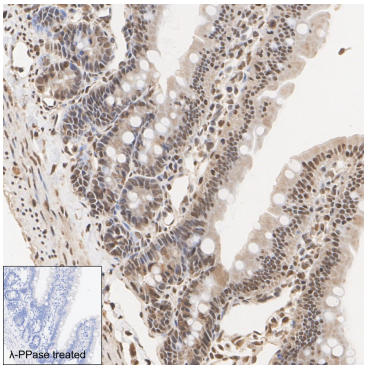

Fig6: Immunohistochemical analysis of paraffin-embedded rat small intestine tissue untreated / treated with λpp with Rabbit anti-Phospho-JunD (S255) antibody (ET1701-35) at 1/200 dilution.

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 (ET1701-35) 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.

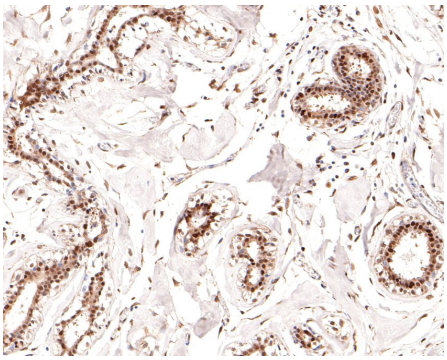

Fig7: Immunohistochemical analysis of paraffin-embedded human breast tissue using anti-Phospho-JunD (S255) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1701-35, 1/100) 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.

Background References

1. Suwei D et al. NLK functions to maintain proliferation and stemness of NSCLC and is a target of metformin. J Hematol Oncol 8:120 (2015).
2. Chen H et al. S100A14: novel modulator of terminal differentiation in esophageal cancer. Mol Cancer Res 11:1542-53 (2013).