Anti-Phospho-c-Jun (T91) Antibody [JJ080-9]

ET1701-32

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP
Molecular Wt:	Predicted band size: 36 kDa
Clone number:	JJ080-9

Description: c-Jun is a protein that in humans is encoded by the JUN gene. c-Jun, in combination with c-Fos, forms the AP-1 early response transcription factor. It was first identified as the Fos-binding protein p39 and only later rediscovered as the product of the JUN gene. c-jun was the first oncogenic transcription factor discovered. The proto-oncogene c-Jun is the cellular homolog of the viral oncoprotein v-jun (P05411). The viral homolog v-jun was discovered in avian sarcoma virus 17 and was named for ju-nana, the Japanese word for 17. The human JUN encodes a protein that is highly similar to the viral protein, which interacts directly with specific target DNA sequences to regulate gene expression. This gene is intronless and is mapped to 1p32-p31, a chromosomal region involved in both translocations and deletions in human malignancies.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Thr91 of Human c-Jun aa 60-109 / 331.

Positive control: A549 treated with 250ng/mL anisomycin for 30 minutes whole cell lysate, NIH/3T3 treated with 250ng/mL anisomycin for 30 minutes whole cell lysate, NIH/3T3 treated with UV for 15 minutes then recover 30 minutes whole cell lysate, A549 cells treated with 250ng/mL anisomycin for 30 minutes, NIH/3T3 cells treated with UV for 15 minutes then recover 30 minutes, human breast carcinoma tissue, human skin tissue, human breast tissue.

Subcellular location: Nucleus.

Database links: SwissProt: P05412 Human | P05627 Mouse | P17325 Rat

Recommended Dilutions:

WB	1:500
IF-Cell	1:500-1:5,000
IF-Tissue	1:50-1:200
IHC-P	1:50-1:200
IP	Use at an assay dependent concentration.

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

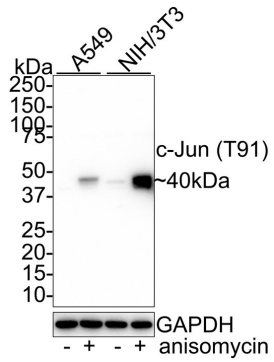

Fig1: Western blot analysis of Phospho-c-Jun (T91) on different lysates with Rabbit anti-Phospho-c-Jun (T91) antibody (ET1701-32) at 1/500 dilution.

Lane 1: A549 whole cell lysate (20 µg/Lane)
Lane 2: A549 treated with 250ng/mL anisomycin for 30 minutes whole cell lysate (20 µg/Lane)
Lane 3: NIH/3T3 whole cell lysate (20 µg/Lane)
Lane 4: NIH/3T3 treated with 250ng/mL anisomycin for 30 minutes whole cell lysate (20 µg/Lane)

Predicted band size: 36 kDa
Observed band size: 40 kDa

Exposure time: 5 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 (ET1701-32) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

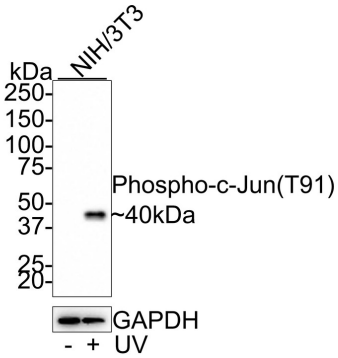

Fig2: Western blot analysis of Phospho-c-Jun (T91) on different lysates with Rabbit anti-Phospho-c-Jun (T91) antibody (ET1701-32) at 1/500 dilution.

Lane 1: NIH/3T3 whole cell lysate
Lane 2: NIH/3T3 treated with UV for 15 minutes then recover 30 minutes whole cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 36 kDa
Observed band size: 40 kDa

Exposure time: 1 minute 30 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 (ET1701-32) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.

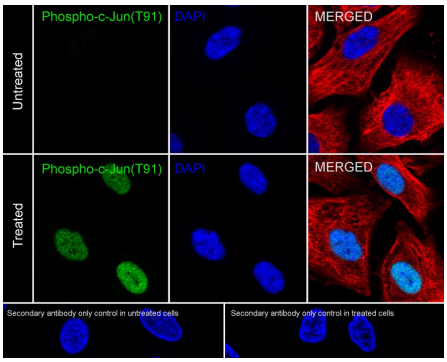

Fig3: Immunocytochemistry analysis of A549 cells untreated / treated with 250ng/mL anisomycin for 30 minutes labeling Phospho-c-Jun (T91) with Rabbit anti-Phospho-c-Jun (T91) antibody (ET1701-32) at 1/500 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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-c-Jun (T91) antibody (ET1701-32) at 1/500 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 (HA601187, 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.

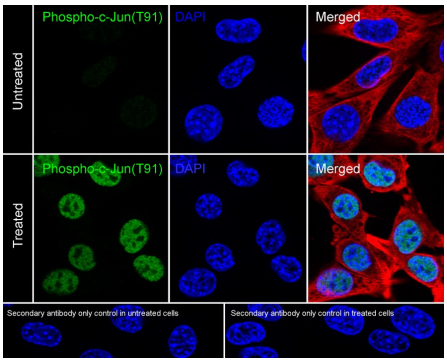

Fig4: Immunocytochemistry analysis of NIH/3T3 cells untreated / treated with UV for 15 minutes then recover 30 minutes labeling Phospho-c-Jun (T91) with Rabbit anti-Phospho-c-Jun (T91) antibody (ET1701-32) at 1/5,000 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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-c-Jun (T91) antibody (ET1701-32) at 1/5,000 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 (HA601187, 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.

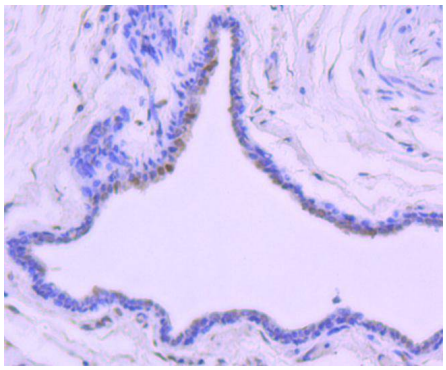

Fig5: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using anti-Phospho-c-Jun (T91) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1701-32, 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.

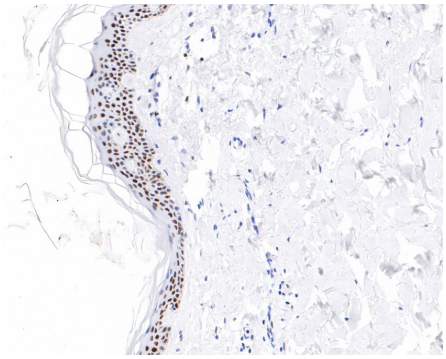

Fig6: Immunohistochemical analysis of paraffin-embedded human skin tissue using anti-Phospho-c-Jun (T91) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1701-32, 1/200) 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.

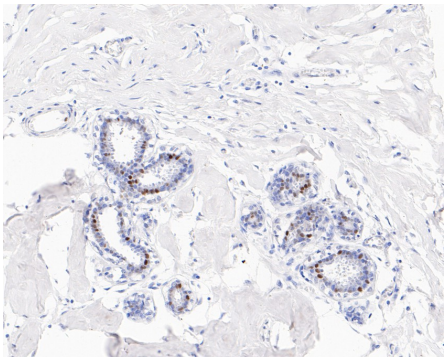

Fig7: Immunohistochemical analysis of paraffin-embedded human breast tissue using anti-Phospho-c-Jun (T91) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1701-32, 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.

Background References

1. Zhang QS et al. Downregulation of SENP1 inhibits cell proliferation, migration and promotes apoptosis in human glioma cells. Oncol Lett 12:217-221 (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).