Anti-Phospho-c-Jun (S73)+JunD (S100) Antibody [JE65-46]

HA722475

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, FC
Molecular Wt:	Predicted band size: 40/45 kDa
Clone number:	JE65-46

Description: Transcription factor that recognizes and binds to the AP-1 consensus motif 5'-TGA[GC]TCA-3'. Heterodimerizes with proteins of the FOS family to form an AP-1 transcription complex, thereby enhancing its DNA binding activity to the AP-1 consensus sequence 5'-TGA[GC]TCA-3' and enhancing its transcriptional activity. Together with FOSB, plays a role in activation-induced cell death of T cells by binding to the AP-1 promoter site of FASLG/CD95L, and inducing its transcription in response to activation of the TCR/CD3 signaling pathway. Promotes activity of NR5A1 when phosphorylated by HIPK3 leading to increased steroidogenic gene expression upon cAMP signaling pathway stimulation. Involved in activated KRAS-mediated transcriptional activation of USP28 in colorectal cancer (CRC) cells. (Microbial infection) Upon Epstein-Barr virus (EBV) infection, binds to viral BZLF1 Z promoter and activates viral BZLF1 expression.

Immunogen: Synthetic phosphopeptide corresponding to residues around Ser73 of human c-Jun.

Positive control: HeLa cell lysate, HeLa treated with 25μg/mL anisomycin for 30 minutes cell lysate, NIH/3T3 cell lysate, NIH/3T3 treated with 25μg/mL anisomycin for 30 minutes cell lysate, RAW264.7 cell lysate, PC-12 cell lysate.

Subcellular location: Nucleus.

Database links: SwissProt: P05412 Human | P17535 Human | P05627 Mouse | P15066 Mouse | P17325 Rat | P52909 Rat

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:100
FC	1:1,000

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

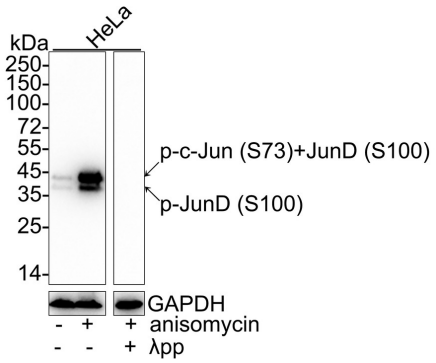

Fig1: Western blot analysis of Phospho-c-Jun (S73)+JunD (S100) on different lysates with Rabbit anti-Phospho-c-Jun (S73)+JunD (S100) antibody (HA722475) at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with 25μg/mL anisomycin for 30 minutes cell lysate
Lane 3: HeLa treated with 25μg/mL anisomycin for 30 minutes cell lysate, then the membrane treated with λpp for 1 hour

Lysates/proteins at 20 μg/Lane.

Predicted band size: 40/45 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 (HA722475) 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.

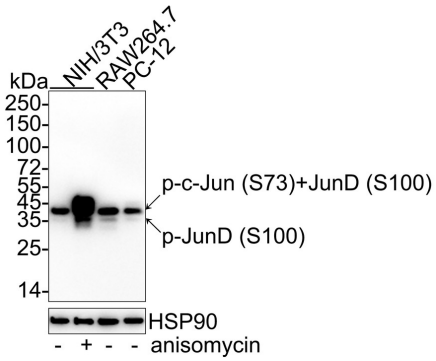

Fig2: Western blot analysis of Phospho-c-Jun (S73)+JunD (S100) on different lysates with Rabbit anti-Phospho-c-Jun (S73)+JunD (S100) antibody (HA722475) at 1/1,000 dilution.

Lane 1: NIH/3T3 cell lysate
Lane 2: NIH/3T3 treated with 25μg/mL anisomycin for 30 minutes cell lysate
Lane 3: RAW264.7 cell lysate
Lane 4: PC-12 cell lysate

Lysates/proteins at 20 μg/Lane.

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

Exposure time: 3 minutes; 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 (HA722475) 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.

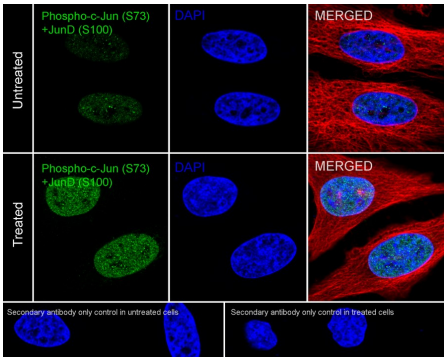

Fig3: Immunocytochemistry analysis of HeLa cells untreated / treated with 250ng/mL anisomycin for 30 minutes labeling Phospho-c-Jun (S73)+JunD (S100) with Rabbit anti-Phospho-c-Jun (S73)+JunD (S100) antibody (HA722475) at 1/100 dilution.

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 (S73)+JunD (S100) antibody (HA722475) 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 (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.

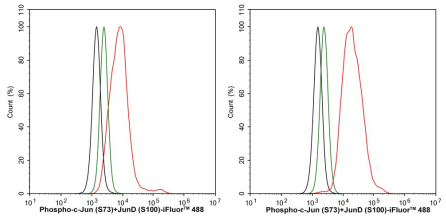

Fig4: Flow cytometric analysis of HeLa cells untreated (left) / treated with 250ng/mL anisomycin for 30 minutes (right) labeling Phospho-c-Jun (S73)+JunD (S100).

Cells were fixed and permeabilized. Then stained with the primary antibody (HA722475, 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).

Background References

1. Qing J., Zhang Y., Derynck R. Structural and functional characterization of the transforming growth factor-beta - induced Smad3/c-Jun transcriptional cooperativity. J. Biol. Chem. 275:38802-38812 (2000)
2. Serra R.W., Fang M., Park S.M., Hutchinson L., Green M.R. A KRAS-directed transcriptional silencing pathway that mediates the CpG island methylator phenotype. Elife 3:E02313-E02313 (2014)