Anti-JunD Antibody [PSH08-85]

HA723037

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC, IP, ChIP
Molecular Wt:	Predicted band size: 35 kDa
Clone number:	PSH08-85

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: Recombinant protein within human JunD aa 1-250.

Positive control: C2C12 cell lysate, NIH/3T3 cell lysate, RAW264.7 cell lysate, C6 cell lysate, HeLa cell lysate, NIH/3T3, C6, mouse brain tissue, rat brain tissue.

Subcellular location: Nucleus.

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

Recommended Dilutions:

WB	1:2,000-1:5,000
IF-Cell	1:100
IHC-P	1:1,000
FC	1:1,000
IP	1-2μg/sample
ChIP	Use 0.5~2 μg for 25 μg of chromatin.

Storage Buffer: PBS (pH7.4), 0.1% 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

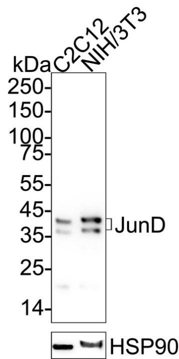

Fig1: Western blot analysis of JunD on different lysates with Rabbit anti-JunD antibody (HA723037) at 1/5,000 dilution.

Lane 1: C2C12 cell lysate
Lane 2: NIH/3T3 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 35 kDa
Observed band size: 39/42 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 (HA723037) at 1/5,000 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.

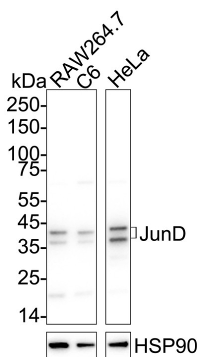

Fig2: Western blot analysis of JunD on different lysates with Rabbit anti-JunD antibody (HA723037) at 1/2,000 dilution.

Lane 1: RAW264.7 cell lysate
Lane 2: C6 cell lysate
Lane 3: HeLa cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 35 kDa
Observed band size: 39/42 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 (HA723037) 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.

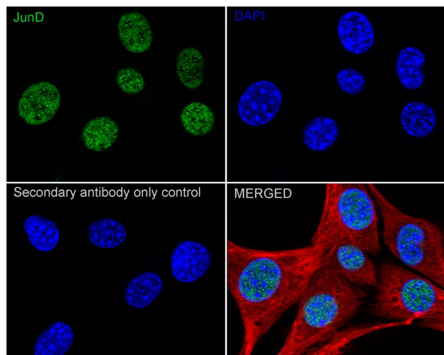

Fig3: Immunocytochemistry analysis of NIH/3T3 cells labeling JunD with Rabbit anti-JunD antibody (HA723037) at 1/100 dilution.

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-JunD antibody (HA723037) 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.

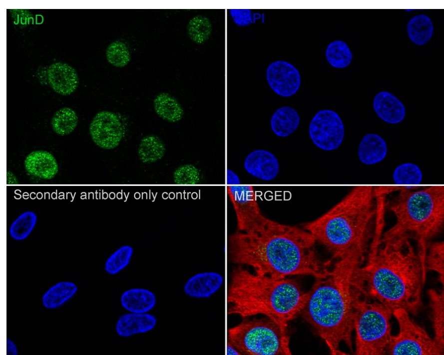

Fig4: Immunocytochemistry analysis of C6 cells labeling JunD with Rabbit anti-JunD antibody (HA723037) at 1/100 dilution.

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-JunD antibody (HA723037) 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.

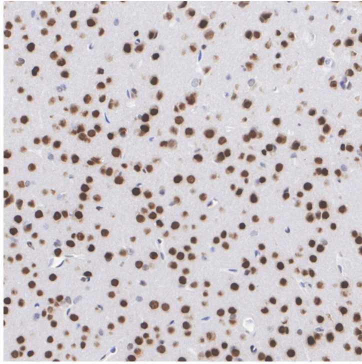

Fig5: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-JunD antibody (HA723037) at 1/1,000 dilution.

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 (HA723037) 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.

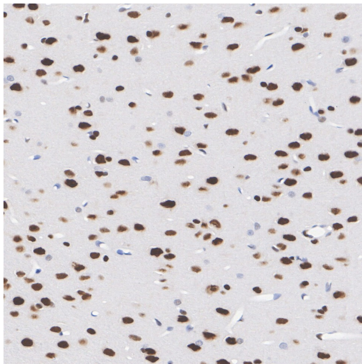

Fig6: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-JunD antibody (HA723037) at 1/1,000 dilution.

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 (HA723037) 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.

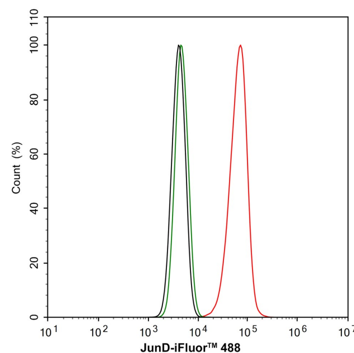

Fig7: Flow cytometric analysis of NIH/3T3 cells labeling JunD.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA723037, 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).

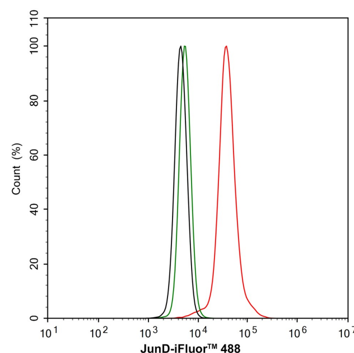

Fig8: Flow cytometric analysis of C6 cells labeling JunD.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA723037, 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).

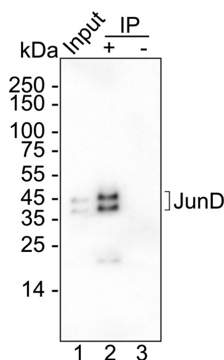

Fig9: JunD was immunoprecipitated from 0.2 mg HeLa cell lysate with HA723037 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA723037 at 1/1,000 dilution. Mouse Anti-Rabbit IgG kappa light chain secondary antibody (M1208-2) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: HA723037 IP in HeLa cell lysate
Lane 3: Rabbit IgG instead of HA723037 in HeLa cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 59 seconds; ECL: K1801

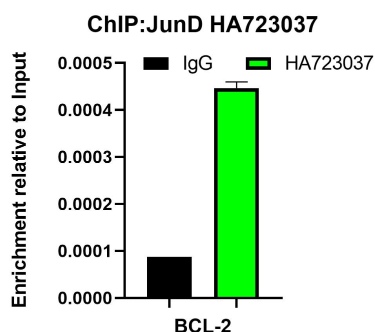

Fig10: Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells with JunD (HA723037) or Normal Rabbit IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated primers. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Wang K et al. JunD Regulates Pancreatic beta-Cells Function by Altering Lipid Accumulation. Front Endocrinol (Lausanne). 2021 Jul

2. Chang Y et al. USP7-mediated JUND suppresses RCAN2 transcription and elevates NFATC1 to enhance stem cell property in colorectal cancer. Cell Biol Toxicol. 2023 Dec