Anti-NFATc3 Antibody [PSH21-27]

HA724210

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse
Applications:	WB, IHC-P, IF-Cell, FC, IP
Molecular Wt:	Predicted band size: 116 kDa
Clone number:	PSH21-27

Description: Nuclear factor of activated T-cells, cytoplasmic 3 is a protein that in humans is encoded by the NFATC3 gene. The product of this gene is a member of the nuclear factors of activated T cells DNA-binding transcription complex. This complex consists of at least two components: a preexisting cytosolic component that translocates to the nucleus upon T cell receptor (TCR) stimulation and an inducible nuclear component. Other members of this family participate to form this complex also. The product of this gene plays a role in the regulation of gene expression in T cells and immature thymocytes. Four transcript variants encoding distinct isoforms have been identified for this gene.

Immunogen: Recombinant protein within human NFATc3 aa 1-450.

Positive control: Jurkat cell lysate, Daudi cell lysate, HeLa cell lysate, Raji cell lysate, NIH/3T3 cell lysate, RAW264.7 cell lysate, RAW264.7 treated with 10μg/mL LPS for 8 hours cell lysate, human thymus tissue, mouse thymus tissue, mouse stomach tissue, K-562, NIH/3T3.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: Q12968 Human | P97305 Mouse

Recommended Dilutions:

WB	1:5,000
IHC-P	1:200
IF-Cell	1:100
FC	1:1,000
IP	1-2μg/sample

Storage Buffer: 1*PBS (pH7.4), 0.1% BSA, 40% Glycerol, 0.2% Proclean 950.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

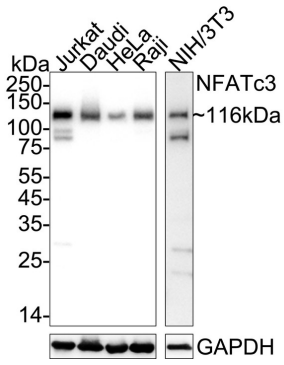


Fig1: Western blot analysis of NFATc3 on different lysates with Rabbit anti-NFATc3 antibody (HA724210) at 1/5,000 dilution.

Lane 1: Jurkat cell lysate
Lane 2: Daudi cell lysate
Lane 3: HeLa cell lysate
Lane 4: Raji cell lysate
Lane 5: NIH/3T3 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 116 kDa
Observed band size: 116 kDa

Exposure time: Lane 1-4: 20 seconds; Lane 5: 1 minute 33 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 (HA724210) 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.

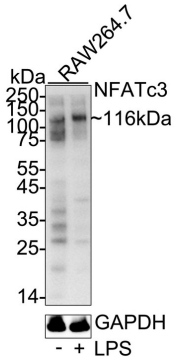


Fig2: Western blot analysis of NFATc3 on different lysates with Rabbit anti-NFATc3 antibody (HA724210) at 1/5,000 dilution.

Lane 1: RAW264.7 cell lysate
Lane 2: RAW264.7 treated with 10µg/mL LPS for 8 hours cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 116 kDa
Observed band size: 116 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 (HA724210) 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.

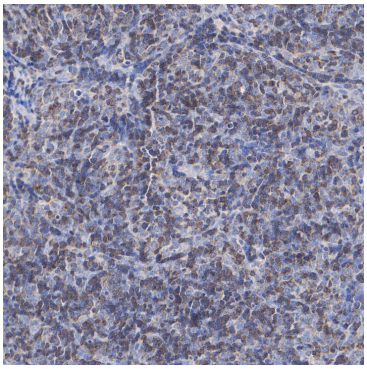


Fig3: Immunohistochemical analysis of paraffin-embedded human thymus tissue with Rabbit anti-NFATc3 antibody (HA724210) at 1/200 dilution.

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 (HA724210) 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.

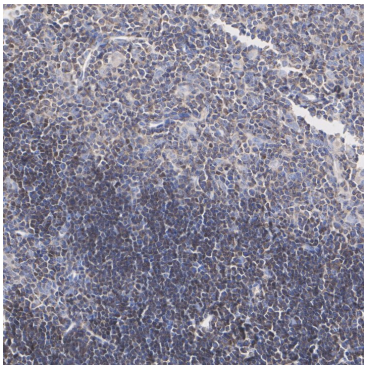


Fig4: Immunohistochemical analysis of paraffin-embedded mouse thymus tissue with Rabbit anti-NFATc3 antibody (HA724210) at 1/200 dilution.

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 (HA724210) 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.

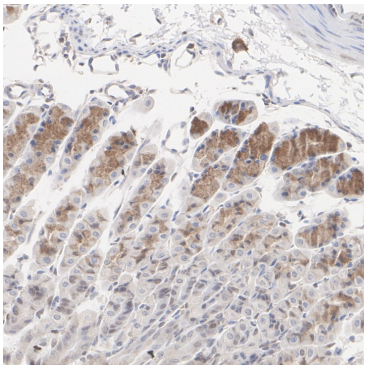


Fig5: Immunohistochemical analysis of paraffin-embedded mouse stomach tissue with Rabbit anti-NFATc3 antibody (HA724210) at 1/200 dilution.

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 (HA724210) 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.

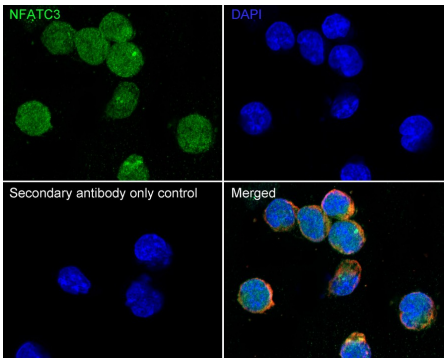


Fig6: Immunocytochemistry analysis of K-562 cells labeling NFATc3 with Rabbit anti-NFATc3 antibody (HA724210) at 1/100 dilution.

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-NFATc3 antibody (HA724210) 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.

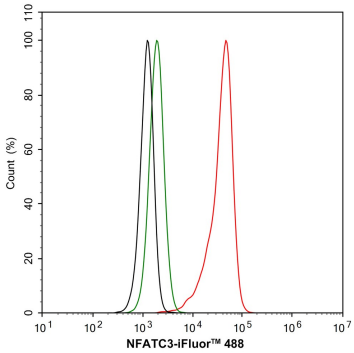


Fig7: Flow cytometric analysis of K-562 cells labeling NFATc3.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA724210, 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).

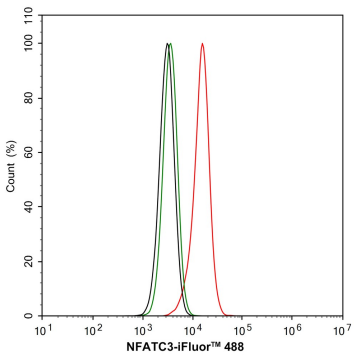


Fig8: Flow cytometric analysis of NIH/3T3 cells labeling NFATc3.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA724210, 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).

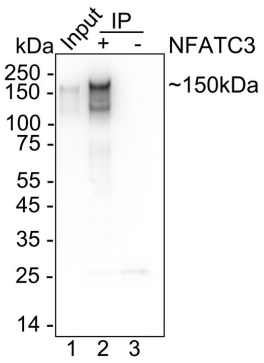


Fig9: NFATc3 was immunoprecipitated from 0.2 mg K-562 cell lysate with HA724210 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA724210 at 1/5,000 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: K-562 cell lysate (input)
Lane 2: HA724210 IP in K-562 cell lysate
Lane 3: Rabbit IgG instead of HA724210 in K-562 cell lysate

Blocking/Dilution buffer: primary antibody dilution (K1803)
Exposure time: 20 seconds; ECL: K1801

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

Background References

1. Liu X et al. Macrophage NFATc3 prevents foam cell formation and atherosclerosis: evidence and mechanisms. Eur Heart J. 2021 Dec
2. Tong Y et al. Hypoxia-induced NFATc3 deSUMOylation enhances pancreatic carcinoma progression. Cell Death Dis. 2022 Apr