Anti-NF-kB p65 Antibody [PSH0-27]

HA721307

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Monkey
Applications:	WB, IHC-P, IF-Cell, FC
Molecular Wt:	Predicted band size: 65 kDa
Clone number:	PSH0-27

Description: Transcription factor p65 also known as nuclear factor NF-kappa-B p65 subunit is a protein that in humans is encoded by the RELA gene. RELA, also known as p65, is a REL-associated protein involved in NF-κB heterodimer formation, nuclear translocation and activation. NF-κB is an essential transcription factor complex involved in all types of cellular processes, including cellular metabolism, chemotaxis, etc. Phosphorylation and acetylation of RELA are crucial post-translational modifications required for NF-κB activation. RELA has also been shown to modulate immune responses, and activation of RELA is positively associated with multiple types of cancer.

Immunogen: Synthetic peptide within human RELA aa 502-551 / 551.

Positive control: HeLa cell lysate, NIH/3T3 cell lysate, PC-12 cell lysate, A431 cell lysate, Raji cell lysate, HL-60 cell lysate, Daudi cell lysate, COS-1 cell lysate, Mouse lung tissue lysate, human breast cancer tissue, human breast tissue, HeLa, NIH/3T3 cells treated with 50μg/mL TNF-α for 20 minutes, PC-12.

Subcellular location: Nucleus, Cytoplasm.

Database links: SwissProt: Q04206 Human | Q04207 Mouse
Entrez Gene: 309165 Rat

Recommended Dilutions:

WB	1:1,000
IHC-P	1:1,000
IF-Cell	1:100-1:200
FC	1:1,000

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

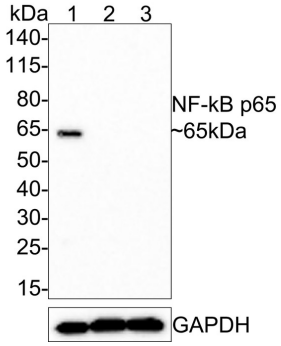


Fig1: Western blot analysis of NF-kB p65 on different lysates with Rabbit anti-NF-kB p65 antibody (HA721307) at 1/500 dilution.

Lane 1: HeLa cell lysate (control) (35 µg/Lane)
Lane 2: HeLa-RELA-KO (1) (35 µg/Lane)
Lane 3: HeLa-RELA-KO (2) (35 µg/Lane)

Predicted band size: 60 kDa
Observed band size: 65 kDa

Exposure time: 3 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 (HA721307) 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.

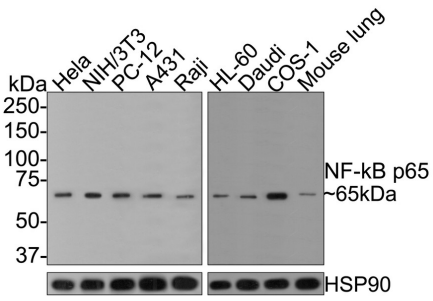


Fig2: Western blot analysis of NF-kB p65 on different lysates with Rabbit anti-NF-kB p65 antibody (HA721307) at 1/500 dilution.

Lane 1: HeLa cell lysate (10 µg/Lane)
Lane 2: NIH/3T3 cell lysate (10 µg/Lane)
Lane 3: PC-12 cell lysate (10 µg/Lane)
Lane 4: A431 cell lysate (10 µg/Lane)
Lane 5: Raji cell lysate (10 µg/Lane)
Lane 6: HL-60 cell lysate (10 µg/Lane)
Lane 7: Daudi cell lysate (10 µg/Lane)
Lane 8: COS-1 cell lysate (10 µg/Lane)
Lane 9: Mouse lung tissue lysate (20 µg/Lane)

Predicted band size: 60 kDa
Observed band size: 65 kDa

Exposure time: 2 minutes;
8% 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 (HA721307) 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 1/300,000 dilution was used for 1 hour at room temperature.

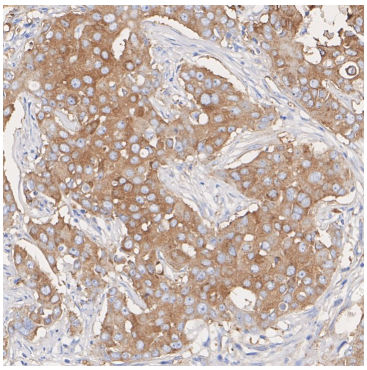


Fig3: Immunohistochemical analysis of paraffin-embedded human breast cancer tissue with Rabbit anti-NF-kB p65 antibody (HA721307) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 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 (HA721307) 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.

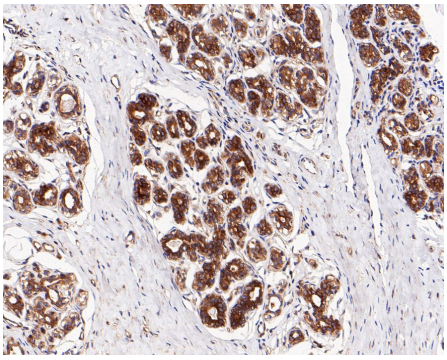
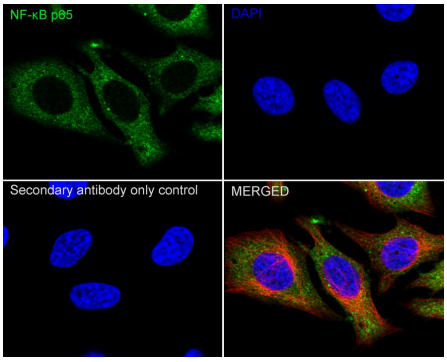


Fig4: Immunohistochemical analysis of paraffin-embedded human breast tissue with Rabbit anti-NF-kB p65 antibody (HA721307) at 1/1,000 dilution.

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 (HA721307) 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.

Fig5: Immunocytochemistry analysis of HeLa cells labeling NF-kB p65 with Rabbit anti-NF-kB p65 antibody (HA721307) at 1/200 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-NF-kB p65 antibody (HA721307) at 1/200 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.

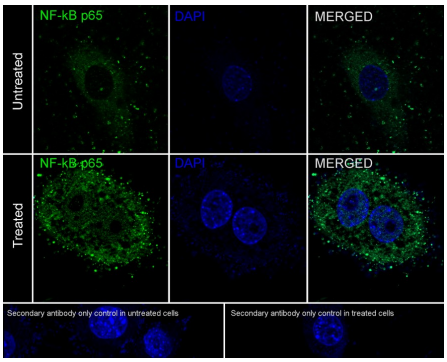


Fig6: Immunocytochemistry analysis of NIH/3T3 cells treated with or without 50μg/mL TNF-α for 20 minutes labeling NF-kB p65 with Rabbit anti-NF-kB p65 antibody (HA721307) at 1/200 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-NF-kB p65 antibody (HA721307) at 1/200 dilution in 2% negative goat serum 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.

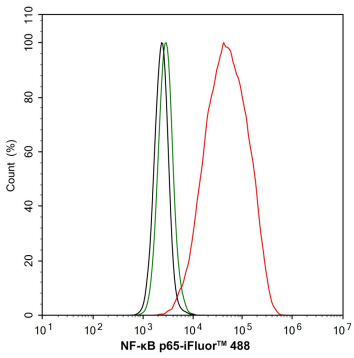


Fig7: Flow cytometric analysis of HeLa cells labeling NF-kB p65.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721307, 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).

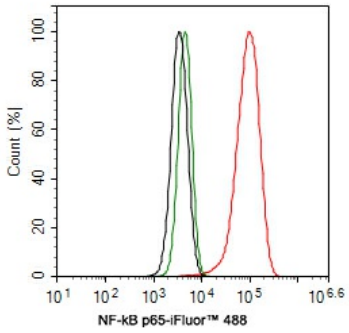


Fig8: Flow cytometric analysis of NIH/3T3 cells labeling NF-kB p65.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721307, 1ug/ml) (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).

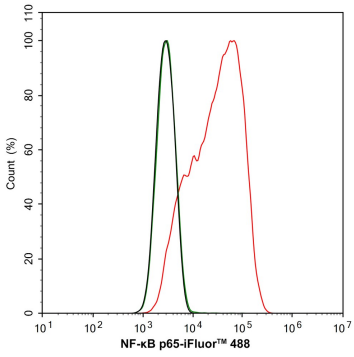


Fig9: Flow cytometric analysis of PC-12 cells labeling NF-kB p65.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721307, 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. Yang B et al. MiR-520b inhibits endothelial activation by targeting NF-κB p65-VCAM1 axis. Biochem Pharmacol. 2021 Jun
2. Sun HJ et al. Polysulfide-mediated sulfhydration of SIRT1 prevents diabetic nephropathy by suppressing phosphorylation and acetylation of p65 NF-κB and STAT3. Redox Biol. 2021 Jan