Anti-Phospho-NF-kB p65 (S536) Antibody [PSH10-58] - BSA and Azide free

HA751361

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell
Molecular Wt:	Predicted band size: 60 kDa
Clone number:	PSH10-58

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 phospho-peptide corresponding to residues surrounding Ser536 of Human NF-kB p65.

Positive control: HeLa treated with 100nM Calyculin A and 20ng/mL TNF-α for 10 minutes cell lysate, NIH/3T3 treated with 100nM Calyculin A and 20ng/mL TNF-α for 10 minutes cell lysate, HeLa cells treated with 100nM Calyculin A and 20ng/mL TNF-α for 15 minutes, C6 treated with 100ng/mL Calyculin A for 1 hour cell lysate, PC-12 treated with 100nM Calyculin A for 30 minutes cell lysate.

Subcellular location: Nucleus, Cytoplasm.

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

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:500

Storage Buffer: PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

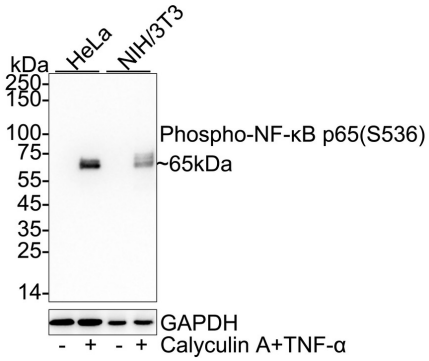

Fig1: Western blot analysis of Phospho-NF-kB p65 (S536) on different lysates with Rabbit anti-Phospho-NF-kB p65 (S536) antibody (HA751361) at 1/5,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)
Lane 2: HeLa treated with 100nM Calyculin A and 20ng/mL TNF-α for 10 minutes cell lysate (20 µg/Lane)
Lane 3: NIH/3T3 cell lysate (20 µg/Lane)
Lane 4: NIH/3T3 treated with 100nM Calyculin A and 20ng/mL TNF-α for 10 minutes cell lysate (20 µg/Lane)

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

Exposure time: 59 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 (HA751361) 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.

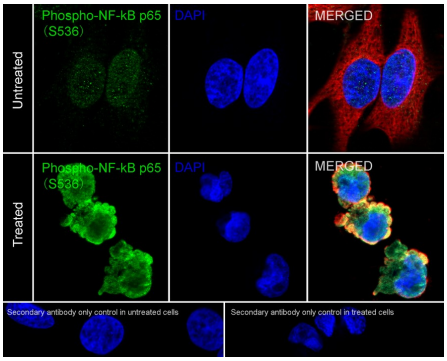

Fig2: Immunocytochemistry analysis of HeLa cells untreated / treated with 100nM Calyculin A and 20ng/mL TNF-α for 15 minutes labeling Phospho-NF-kB p65 (S536) with Rabbit anti-Phospho-NF-kB p65 (S536) antibody (HA751361) at 1/500 dilution.

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-NF-kB p65 (S536) antibody (HA751361) 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.

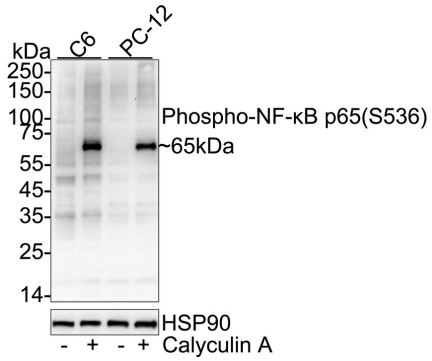

Fig3: Western blot analysis of Phospho-NF-kB p65 (S536) on different lysates with Rabbit anti-Phospho-NF-kB p65 (S536) antibody (HA751361) at 1/5,000 dilution.

Lane 1: C6 cell lysate (20 µg/Lane)
Lane 2: C6 treated with 100ng/mL Calyculin A for 1 hour cell lysate (20 µg/Lane)
Lane 3: PC-12 cell lysate (20 µg/Lane)
Lane 4: PC-12 treated with 100nM Calyculin A for 30 minutes cell lysate (20 µg/Lane)

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

Exposure time: 59 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 (HA751361) 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.

Background References

1. Shim H et al. NF-kappaB p65 represses microRNA-124 transcription in diffuse large B-cell lymphoma. Genes Genomics. 2020 May
2. Ren C et al. Ubiquitination of NF-kappaB p65 by FBXW2 suppresses breast cancer stemness, tumorigenesis, and paclitaxel resistance. Cell Death Differ. 2022 Feb