# Anti-NF-kB p65 Antibody

## HA500402

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| **Product Type:** | Rabbit polyclonal IgG, primary antibodies |
| **Species reactivity:** | Human, Mouse, Rat |
| **Applications:** | WB, IHC-P, IF-Cell, FC, IP |
| **Molecular Wt:** | Predicted band size: 65 kDa |

**Description:** NF-kappa-B is a pleiotropic transcription factor present in almost all cell types and is the endpoint of a series of signal transduction events that are initiated by a vast array of stimuli related to many biological processes such as inflammation, immunity, differentiation, cell growth, tumorigenesis and apoptosis. NF-kappa-B is a homo- or heterodimeric complex formed by the Rel-like domain-containing proteins RELA/p65, RELB, NFKB1/p105, NFKB1/p50, REL and NFKB2/p52. The heterodimeric RELA-NFKB1 complex appears to be most abundant one. The dimers bind at kappa-B sites in the DNA of their target genes and the individual dimers have distinct preferences for different kappa-B sites that they can bind with distinguishable affinity and specificity. Different dimer combinations act as transcriptional activators or repressors, respectively. The NF-kappa-B heterodimeric RELA-NFKB1 and RELA-REL complexes, for instance, function as transcriptional activators. NF-kappa-B is controlled by various mechanisms of post-translational modification and subcellular compartmentalization as well as by interactions with other cofactors or corepressors. NF-kappa-B complexes are held in the cytoplasm in an inactive state complexed with members of the NF-kappa-B inhibitor (I-kappa-B) family. In a conventional activation pathway, I-kappa-B is phosphorylated by I-kappa-B kinases (IKKs) in response to different activators, subsequently degraded thus liberating the active NF-kappa-B complex which translocates to the nucleus. The inhibitory effect of I-kappa-B on NF-kappa-B through retention in the cytoplasm is exerted primarily through the interaction with RELA. RELA shows a weak DNA-binding site which could contribute directly to DNA binding in the NF-kappa-B complex. Beside its activity as a direct transcriptional activator, it is also able to modulate promoters accessibility to transcription factors and thereby indirectly regulate gene expression. Associates with chromatin at the NF-kappa-B promoter region via association with DDX1. Essential for cytokine gene expression in T-cell.

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| **Immunogen:** | Synthetic peptide within human RELA aa 450-550. |
| **Positive control:** | HeLa cell lysate, MCF7 cell lysate, C2C12 cell lysate, RAW264.7 cell lysate, PC-12 cell lysate, C6 cell lysate, SH-SY5Y, PC-12, human breast tissue. |
| **Subcellular location:** | Nucleus, Cytoplasm. |
| **Database links:** | SwissProt: Q04206 Human \| Q04207 Mouse<br>Entrez Gene: 309165 Rat |

**Recommended Dilutions:**

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| **WB** | 1:50,000 |
| **IHC-P** | 1:200 |
| **IF-Cell** | 1:100-1:200 |
| **FC** | 1:1,000 |
| **IP** | 1-2μg/sample |

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| **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:** | Immunogen affinity purified. |

## Images

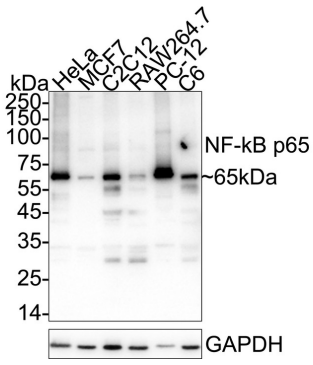


**Fig1:** Western blot analysis of NF-kB p65 on different lysates with Rabbit anti-NF-kB p65 antibody (HA500402) at 1/50,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: MCF7 cell lysate
Lane 3: C2C12 cell lysate
Lane 4: RAW264.7 cell lysate
Lane 5: PC-12 cell lysate
Lane 6: C6 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 65 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 (HA500402) at 1/50,000 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.

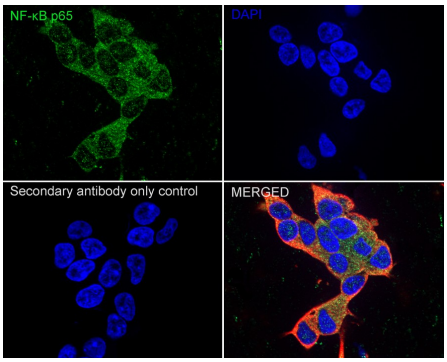


**Fig2:** Immunocytochemistry analysis of SH-SY5Y cells labeling NF-kB p65 with Rabbit anti-NF-kB p65 antibody (HA500402) at 1/100 dilution.

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-NF-kB p65 antibody (HA500402) 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.

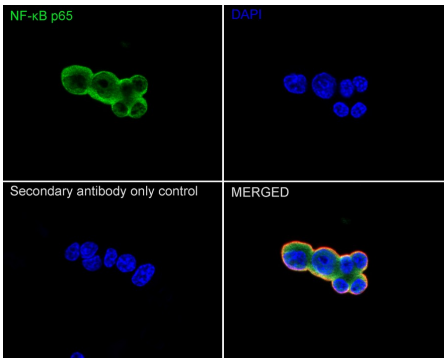


**Fig3:** Immunocytochemistry analysis of PC-12 cells labeling NF-kB p65 with Rabbit anti-NF-kB p65 antibody (HA500402) at 1/200 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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 (HA500402) 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 (M1305-2, 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.

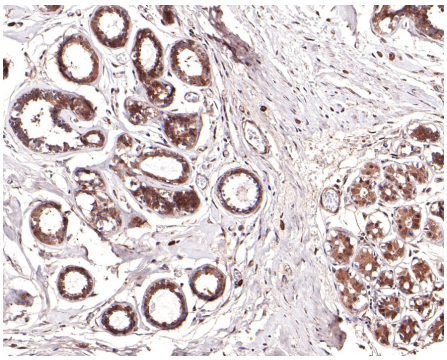

**Fig4:** Immunohistochemical analysis of paraffin-embedded human breast tissue with Rabbit anti-NF-kB p65 antibody (HA500402) at 1/200 dilution.

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 dd$H_2O$ and PBS, and then probed with the primary antibody (HA500402) 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.

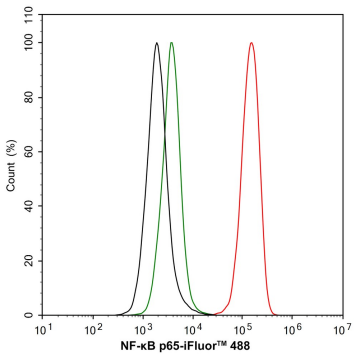


**Fig5:** Flow cytometric analysis of SH-SY5Y cells labeling NF-kB p65.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA500402, 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).

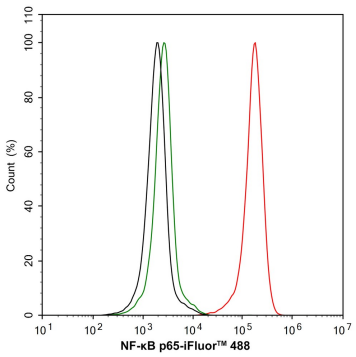


**Fig6:** Flow cytometric analysis of PC-12 cells labeling NF-kB p65.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA500402, 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).

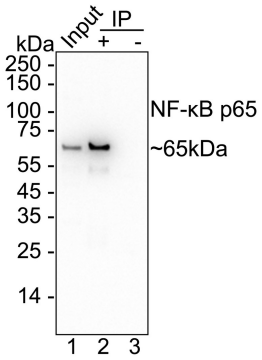


**Fig7:** NF-kB p65 was immunoprecipitated from 0.2 mg HeLa cell lysate with HA500402 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA500402 at 1/5,000 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: HA500402 IP in HeLa cell lysate
Lane 3: Rabbit IgG instead of HA500402 in HeLa cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 7 seconds; ECL: K1801



## Background References

1. "Breast cancer metastasis suppressor 1 functions as a corepressor by enhancing histone deacetylase 1-mediated deacetylation of RelA/p65 and promoting apoptosis." Liu Y., Smith P.W., Jones D.R. Mol. Cell. Biol. 26:8683-8696(2006)
2. "SIRT2 regulates NF-kappaB dependent gene expression through deacetylation of p65 Lys310." Rothgiesser K.M., Erener S., Waibel S., Luscher B., Hottiger M.O. J. Cell Sci. 123:4251-4258(2010)