# Anti-NF-kB p65 Antibody

## ER0815



Product Type: Species reactivity: Applications: Molecular Wt:	Rabbit polyclonal IgG, primary antibodies Human, Mouse, Rat, Zebrafish WB, IHC-P, FC Predicted band size: 60 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 and the heterodimeric p65-p50 complex appears to be most abundant one. In unstimulated cells, NF-κB is sequestered in the cytoplasm by IκB inhibitory proteins. NF-κB-activating agents can induce the phosphorylation of IκB proteins, targeting them for rapid degradation through the ubiquitin-proteasome pathway and releasing NF-κB to enter the nucleus where it regulates gene expression.
lmmunogen:	Synthetic peptide within N-terminal human RELA.
Positive control:	Hela cell lysate, A549 cell lysate, PC12 cell lysate, Mouse embryonic stem cell lysate, NIH/3T3 cell lysate, zebrafish lysates, human lung cancer tissue, human lung tissue, human spleen tissue, mouse spleen tissue, rat spleen tissue, zebrafish, Hela.
Subcellular location:	Nucleus, cytoplasm.
Database links:	SwissProt: Q04206 Human   Q04207 Mouse Entrez Gene: 309165 Rat
Recommended Dilutions: WB IHC-P FC	1:1,000-1:2,000 1:200 1:50-1:100
Storage Buffer:	1*PBS (pH7.4), 0.2% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
Storage Instruction:	Shipped at 4 $^\circ\!\mathrm{C}$ . Store at +4 $^\circ\!\mathrm{C}$ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20 $^\circ\!\mathrm{C}$ long term.
Purity:	Immunogen affinity purified.

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#### Images



**Fig1:** Western blot analysis of NF-κB p65 on different lysates using anti-NF-κB p65 antibody at 1/1,000 dilution. **Positive control:** Lane 1: Hela cell lysate Lane 2: A549 cell lysate Lane 3: PC12 cell lysate Lane 4: Mouse embryonic stem cell lysate

- Lane 5: NIH/3T3 cell lysate

**Fig2:** Western blot analysis of NF-kB p65 on zebrafish lysates with Rabbit anti-NF-kB p65 antibody (ER0815) at 1/2,000 dilution.

Lysates at 20 µg/Lane.

Predicted band size: 60kDa Observed band size: 70 kDa

Exposure time: 3min20s;

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 (ER0815) at 1/2,000 dilution was used in 5% NFDM/TBST at  $4^{\circ}$ C 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 lung cancer tissue with Rabbit anti-NF-kB p65 antibody (ER0815) 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 ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ER0815) 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.

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**Fig4:** Immunohistochemical analysis of paraffin-embedded human lung tissue with Rabbit anti-NF-kB p65 antibody (ER0815) 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 ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ER0815) 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 human spleen tissue with Rabbit anti-NF-kB p65 antibody (ER0815) 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 ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ER0815) 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:** Immunohistochemical analysis of paraffin-embedded mouse spleen tissue with Rabbit anti-NF-kB p65 antibody (ER0815) 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 ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ER0815) 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.

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**Fig7:** Immunohistochemical analysis of paraffin-embedded rat spleen tissue with Rabbit anti-NF-kB p65 antibody (ER0815) 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 ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ER0815) 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.



**Fig8:** Immunohistochemical analysis of paraffin-embedded zebrafish with Rabbit anti-NF-kB p65 antibody (ER0815) 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 ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ER0815) 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.



**Fig9:** Flow cytometric analysis of Hela cells with NF- $\kappa$ B p65 antibody at 1/50 dilution (blue) compared with an unlabelled control (cells without incubation with primary antibody; red). Goat anti rabbit IgG (FITC) was used as the secondary antibody.

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

#### **Background References**

- 1. "Duration of nuclear NF-kappaB action regulated by reversible acetylation." Chen L.F., Fischle W., Verdin E., Greene W.C.Science 293:1653-1657(2001)
- "A novel protein overexpressed in hepatoma accelerates export of NF-kappa B from the nucleus and inhibits p53dependent apoptosis." Higashitsuji H., Higashitsuji H., Nagao T., Nonoguchi K., Fujii S., Itoh K., Fujita J. Cancer Cell 2:335-346(2002)
- "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)
- 4. "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)

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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

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