Anti-NF-kB p65 Antibody [SZ10-04]

ET1603-12

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Zebrafish
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP, FC
Molecular Wt:	Predicted band size: 65 kDa
Clone number:	SZ10-04

Description: Proteins encoded by the v-Rel viral oncogene and its cellular homolog, c-Rel, are members of a family of transcription factors that include the two subunits of the transcription factor NF kB (p50 and p65) and the Drosophila maternal morphogen, dorsal. Both proteins specifically bind to DNA sequences that are the same or slight variations of the 10 bp kB sequence in the immunoglobulin k light chain enhancer. This same sequence is also present in a number of other cellular and viral enhancers. The DNA binding activity of NFkB is activated and NFkB is subsequently transported from the cytoplasm to the nucleus in cells exposed to mitogens or growth factors. cDNAs encoding precursors for two distinct proteins of the same size have been described, designated p105 and p100. The p105 precursor contains p50 at its N-terminus and a C-terminal region that when expressed as a separate molecule, designated pdI, binds to p50 and regulates its activity.

Immunogen: Synthetic peptide within human RELA aa 490-540.

Positive control: A549 cell lysate, MCF7 cell lysate, HeLa cell lysate, RAW264.7 cell lysate, HeLa, human lung squamous cell carcinoma tissue, human lung cancer tissue, human lung tissue, human spleen tissue, mouse spleen tissue, rat spleen tissue.

Subcellular location: Nucleus, Cytoplasm.

Database links: SwissProt: Q04206 Human | Q04207 Mouse

Recommended Dilutions:

WB	1:5,000-1:10,000
IF-Cell	1:100-1:400
IF-Tissue	1:100-1:200
IHC-P	1:200-1:500
FC	1:2,000
IP	Use at an assay dependent concentration.

Storage Buffer: 1*TBS (pH7.4), 0.05% 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

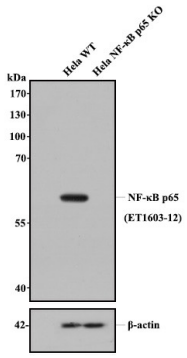

Fig1: Western blot analysis of NF-kB p65 on different lysates with Rabbit anti-NF-kB p65 antibody (ET1603-12) at 1/5,000 dilution.

Lane 1: Wild-type HeLa cell lysate
Lane 2: HeLa KO cell lysate

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 10 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 (ET1603-12) 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.

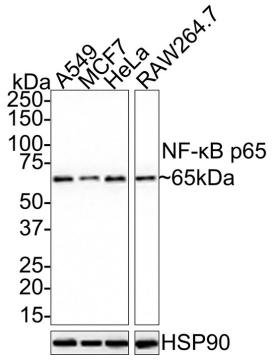

Fig2: Western blot analysis of NF-kB p65 on different lysates with Rabbit anti-NF-kB p65 antibody (ET1603-12) at 1/5,000 dilution.

Lane 1: A549 cell lysate
Lane 2: MCF7 cell lysate
Lane 3: HeLa cell lysate
Lane 4: RAW264.7 cell lysate

Lysates/proteins at 15 µg/Lane.

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

Exposure time: 49 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 (ET1603-12) at 1/5,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

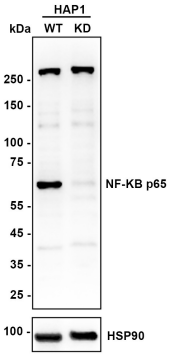

Fig3: Western blot analysis of NF-kB p65 on different lysates with Rabbit anti-NF-kB p65 antibody (ET1603-12) at 1/5,000 dilution.

Lane 1: HAP1-parental cell lysate
Lane 2: HAP1-NF-kB p65 KD cell lysate

Lysates/proteins at 10 µg/Lane.

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

Exposure time: 60 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 (ET1603-12) at 1/5,000 dilution was used in 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.

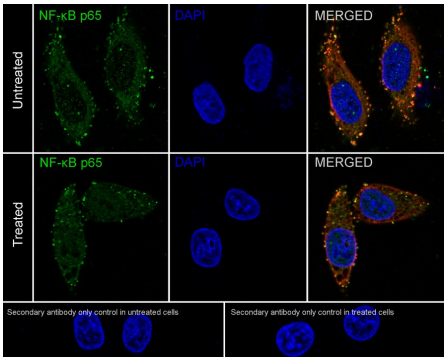

Fig4: Immunocytochemistry analysis of HeLa cells treated with or without 50 ng/mL TNF-alpha for 20 minutes labeling NF-kB p65 with Rabbit anti-NF-kB p65 antibody (ET1603-12) at 1/400 dilution.

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 (ET1603-12) at 1/400 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. 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.

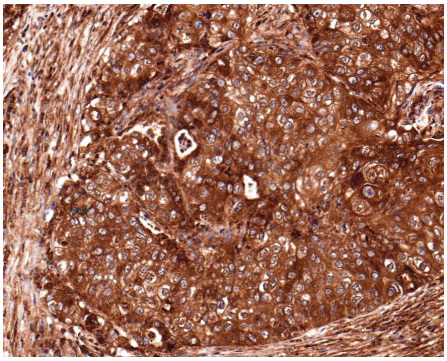

Fig5: Immunohistochemical analysis of paraffin-embedded human lung squamous cell carcinoma tissue with Rabbit anti-NF-kB p65 antibody (ET1603-12) at 1/400 dilution.

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 (ET1603-12) at 1/400 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.

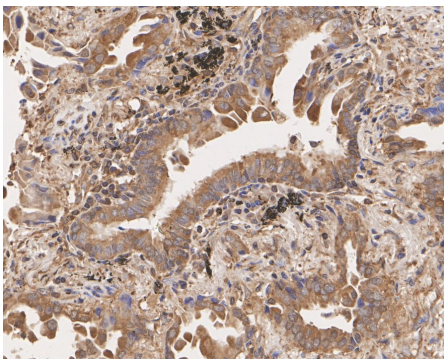

Fig6: Immunohistochemical analysis of paraffin-embedded human lung cancer tissue with Rabbit anti-NF-kB p65 antibody (ET1603-12) at 1/200 dilution.

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_2O and PBS, and then probed with the primary antibody (ET1603-12) 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.

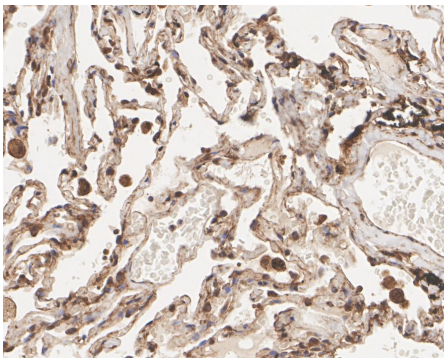

Fig7: Immunohistochemical analysis of paraffin-embedded human lung tissue with Rabbit anti-NF-kB p65 antibody (ET1603-12) at 1/200 dilution.

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_2O and PBS, and then probed with the primary antibody (ET1603-12) 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.

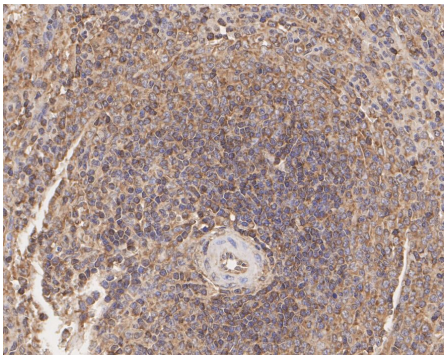

Fig8: Immunohistochemical analysis of paraffin-embedded human spleen tissue with Rabbit anti-NF-kB p65 antibody (ET1603-12) at 1/200 dilution.

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_2O and PBS, and then probed with the primary antibody (ET1603-12) 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.

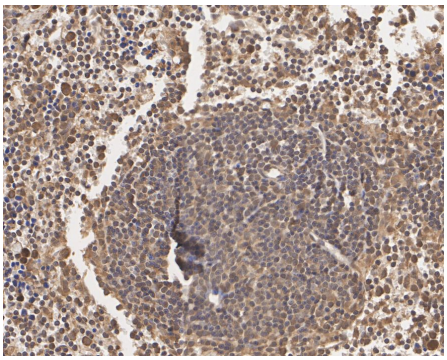

Fig9: Immunohistochemical analysis of paraffin-embedded mouse spleen tissue with Rabbit anti-NF-kB p65 antibody (ET1603-12) at 1/200 dilution.

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_2O and PBS, and then probed with the primary antibody (ET1603-12) 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.

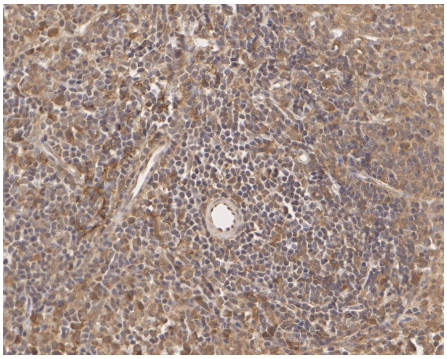

Fig10: Immunohistochemical analysis of paraffin-embedded rat spleen tissue with Rabbit anti-NF-kB p65 antibody (ET1603-12) at 1/200 dilution.

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_2O and PBS, and then probed with the primary antibody (ET1603-12) 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.

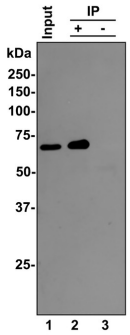

Fig11: NF-κB p65 was immunoprecipitated from 0.5 mg Hela whole cell lysates with ET1603-12 at 2 µg/mL. Western blot was performed from the immunoprecipitate using ET1603-12 at 1/500 dilution for 45 minutes at room temperature. Goat anti-Rabbit IgG-HRP Secondary Antibody (HA1001) was used at 1:300,000 dilution for 30 minutes at room temperature.

Lane 1: Hela whole cell lysates at 10 µg;
Lane 2: NF-κB p65 (ET1603-12) IP in Hela whole cell lysates;
Lane 3: Rabbit IgG instead of NF-κB p65 (ET1603-12) in Hela whole cell lysates.

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

Exposure time: 10 seconds;

8% SDS-PAGE gel.

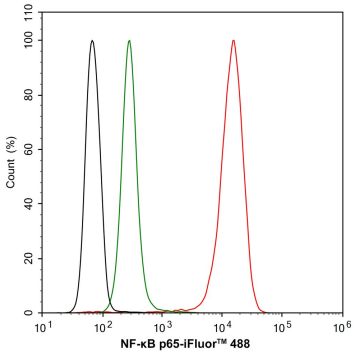

Fig12: Flow cytometric analysis of HeLa cells labeling NF-kB p65.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1603-12, red) at 1/2,000 dilution, 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).

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

Background References

1. Kang K et al. Carnosic acid slows photoreceptor degeneration in the Pde6b(rd10) mouse model of retinitis pigmentosa. Sci Rep 6:22632 (2016).
2. Kropp KA et al. A temporal gate for viral enhancers to co-opt Toll-like-receptor transcriptional activation pathways upon acute infection. PLoS Pathog 11:e1004737 (2015).