Anti-NF-kB p105 / p50 Antibody [SZ20-01]

ET1603-18

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, ICC, FC, IF-Tissue
Molecular Wt:	Predicted band size: 105/50 kDa
Clone number:	SZ20-01

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 NFkB (p50 and p65) and the Drosophila maternal morphagen, dorsal. These proteins share sequence homology over a region of 300 amino acids at their NH2-terminus, the region that contains their DNA binding and dimerization domains. The DNA binding activity of NF B is activated and rapidly transported from the cytoplasm to the nucleus in cells exposed to mitogens or growth factors. cDNAs encoding precursors for two distinct proteins have been described. These proteins, designated p105 and p100, are highly related but map on different chromosomes. The p105 (p110) 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 NFKB1 aa 330-370.

Positive control: THP-1 cell lysate, Raji cell lysate, Daudi cell lysate, MCF7 cell lysate, NIH/3T3 cell lysate, Hela cell lysate, PC-12 cell lysate, human tonsil tissue, human spleen tissue, mouse bladder tissue, mouse prostate tissue.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: P19838 Human | P25799 Mouse | Q63369 Rat

Recommended Dilutions:

WB	1:5,000
IHC-P	1:200-1:1,000
ICC	1:100
FC	1:1,000
IF-Tissue	1:50-1:200

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

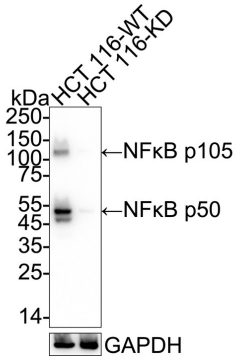

Fig1: Western blot analysis of NF-kB p105 / p50 on different lysates with Rabbit anti-NF-kB p105 / p50 antibody (ET1603-18) at 1/5,000 dilution.

Lane 1: HCT 116-si NT cell lysate
Lane 2: HCT 116-si NFKB1 cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 105/50 kDa
Observed band size: 105/50 kDa

Exposure time: 30 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 (ET1603-18) 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.

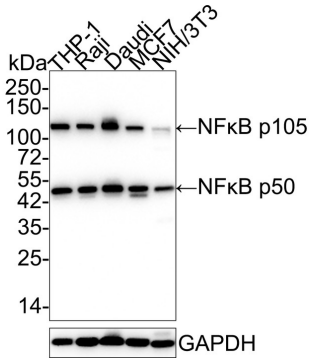

Fig2: Western blot analysis of NF-kB p105 / p50 on different lysates with Rabbit anti-NF-kB p105 / p50 antibody (ET1603-18) at 1/5,000 dilution.

Lane 1: THP-1 cell lysate
Lane 2: Raji cell lysate
Lane 3: Daudi cell lysate
Lane 4: MCF7 cell lysate
Lane 5: NIH/3T3 cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 105/50 kDa
Observed band size: 105/50 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 (ET1603-18) 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.

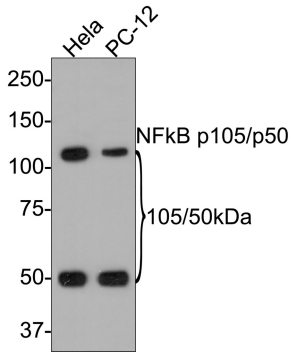

Fig3: Western blot analysis of NF-kB p105 / p50 on different lysates with Rabbit anti-NF-kB p105 / p50 antibody (ET1603-18) at 1/500 dilution.

Lane 1: Hela cell lysate
Lane 2: PC-12 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 50/105 kDa
Observed band size: 50/105 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 (ET1603-18) 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.

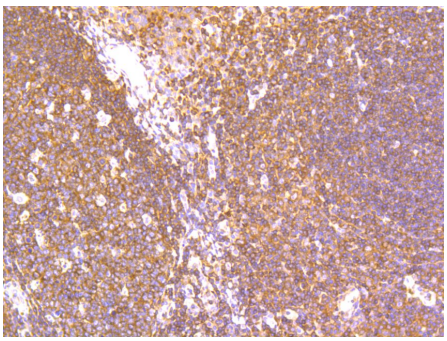

Fig4: Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-NF-kB p105 / p50 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1603-18, 1/200) for 30 minutes 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.

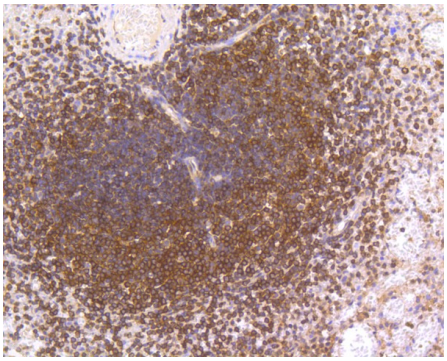

Fig5: Immunohistochemical analysis of paraffin-embedded human spleen tissue using anti-NF-kB p105 / p50 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1603-18, 1/200) for 30 minutes 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.

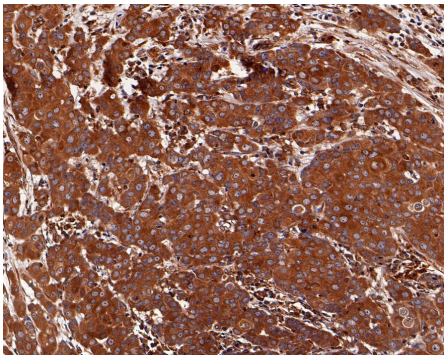

Fig6: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue with Rabbit anti-NF-kB p105 / p50 antibody (ET1603-18) at 1/1,000 dilution.

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 (ET1603-18) 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.

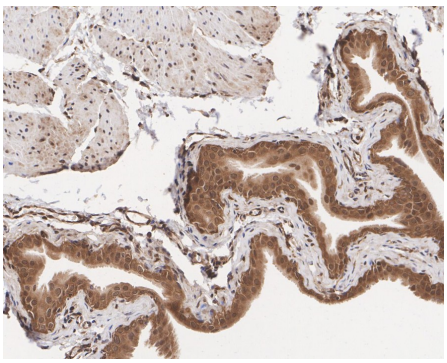

Fig7: Immunohistochemical analysis of paraffin-embedded rat bladder tissue with Rabbit anti-NF-kB p105 / p50 antibody (ET1603-18) at 1/1,000 dilution.

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 (ET1603-18) 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.

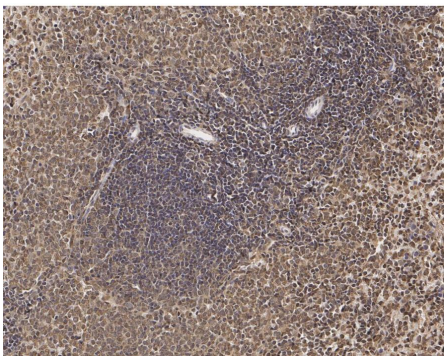

Fig8: Immunohistochemical analysis of paraffin-embedded rat spleen tissue with Rabbit anti-NF-kB p105 / p50 antibody (ET1603-18) at 1/200 dilution.

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 (ET1603-18) 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."

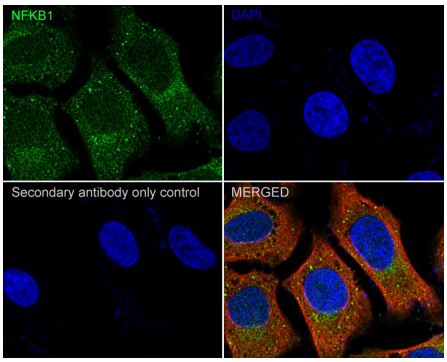

Fig9: Immunocytochemistry analysis of HeLa cells labeling NF-kB p105 / p50 with Rabbit anti-NF-kB p105 / p50 antibody (ET1603-18) at 1/100 dilution.

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 p105 / p50 antibody (ET1603-18) 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 (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.

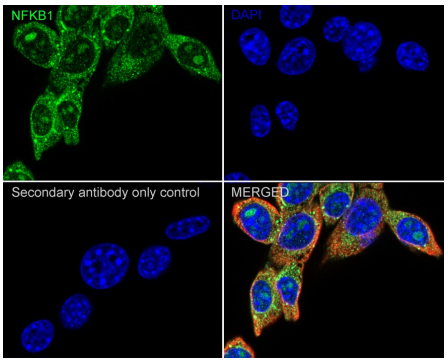

Fig10: Immunocytochemistry analysis of NIH/3T3 cells labeling NF-kB p105 / p50 with Rabbit anti-NF-kB p105 / p50 antibody (ET1603-18) at 1/100 dilution.

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 p105 / p50 antibody (ET1603-18) 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 (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.

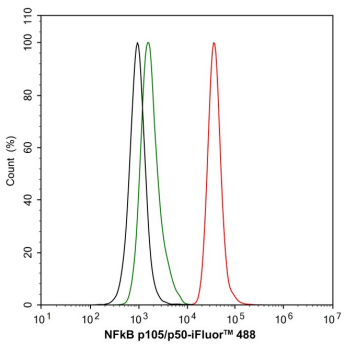

Fig11: Flow cytometric analysis of HeLa cells labeling NF-kB p105 / p50.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1603-18, 1μg/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).

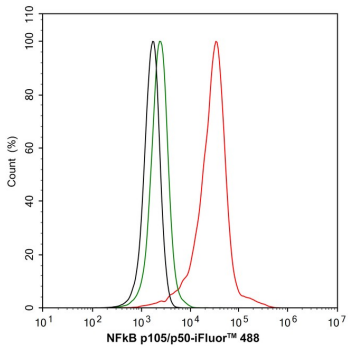

Fig12: Flow cytometric analysis of NIH/3T3 cells labeling NF-kB p105 / p50.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1603-18, 1μg/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)."

Background References

1. Liu Z et al. Mark4 promotes oxidative stress and inflammation via binding to PPAR and activating NF-kB pathway in mice adipocytes. Sci Rep 6:21382 (2016).
2. Lin JJ et al. Toll-like receptor 4 signaling in neurons of trigeminal ganglion contributes to nociception induced by acute pulpitis in rats. Sci Rep 5:12549 (2015).