Anti-IKB alpha Antibody [SZ00-07]

ET1603-6

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP, FC
Molecular Wt:	Predicted band size: 36 kDa
Clone number:	SZ00-07

Description: NFKB1 or NFKB2 is bound to REL, RELA, or RELB to form the NFKB complex. The NFKB complex is inhibited by I-kappa-B proteins, which inactivate NF-kappa-B by trapping it in the cytoplasm. Phosphorylation of serine residues on the I-kappa-B proteins by kinases marks them for destruction via the ubiquitination pathway, thereby allowing activation of the NF-kappa-B complex. Activated NFKB complex translocates into the nucleus and binds DNA at kappa-B-binding motifs such as 5-prime GGGRNNYYCC 3-prime or 5-prime HGGARNYYCC 3-prime.

Immunogen: Synthetic peptide within human IKB alpha aa 1-50.

Positive control: HeLa cell lysate, SK-Br-3 cell lysate, NIH/3T3 cell lysate, RAW264.7 cell lysate, PC-12 cell lysate, Hela, MCF-7, SHG-44, human kidney tissue, mouse kidney tissue, mouse brain tissue, mouse stomach tissue.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: P25963 Human | Q9Z1E3 Mouse | Q63746 Rat

Recommended Dilutions:

WB	1:5,000-1:20,000
IF-Cell	1:100-1:200
IF-Tissue	1:200-1:500
IHC-P	1:200-1:1,000
IP	Use at an assay dependent concentration.
FC	1:1,000

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

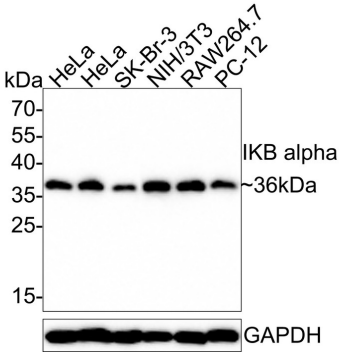

Fig1: Western blot analysis of IKB alpha on different lysates with Rabbit anti-IKB alpha antibody (ET1603-6) at 1/5,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa cell lysate
Lane 3: SK-Br-3 cell lysate
Lane 4: NIH/3T3 cell lysate
Lane 5: RAW264.7 cell lysate
Lane 6: PC-12 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 36 kDa
Observed band size: 36 kDa

Exposure time: 1 minute;

12% 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 (ET1603-6) 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 1:300,000 dilution was used for 1 hour at room temperature.

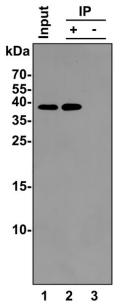

Fig2: IKB alpha was immunoprecipitated from 0.5 mg Hela whole cell lysates with ET1603-6 at 2 µg/mL. Western blot was performed from the immunoprecipitate using ET1603-6 at 1/5,000 dilution for 45 minutes at room temperature. Goat anti-Rabbit IgG-HRP Secondary Antibody (HA1001) was used at 1:300,000 dilution for 30 minutes at room temperature.

Lane 1: Hela whole cell lysates at 10 µg;
Lane 2: IKB alpha (ET1603-6) IP in Hela whole cell lysates;
Lane 3: Rabbit IgG instead of IKB alpha (ET1603-6) in Hela whole cell lysates.

Predicted band size: 36 kDa
Observed band size: 36 kDa

Exposure time: 1 minute;

12% SDS-PAGE gel.

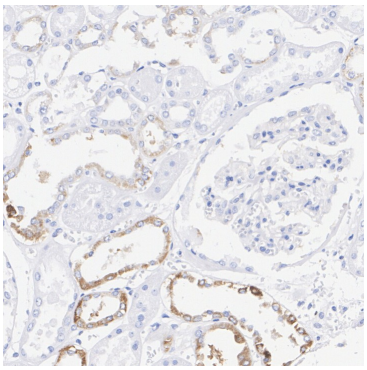

Fig3: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-IKB alpha antibody (ET1603-6) at 1/1,000 dilution.

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 (ET1603-6) 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.

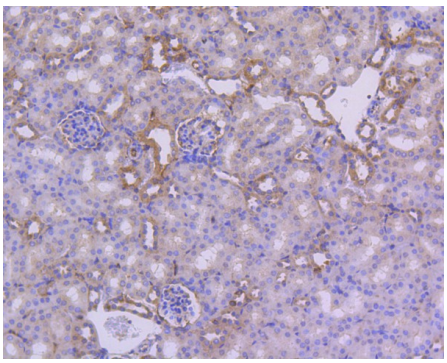

Fig4: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-IKB alpha antibody (ET1603-6) at 1/200 dilution.

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 (ET1603-6) 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.

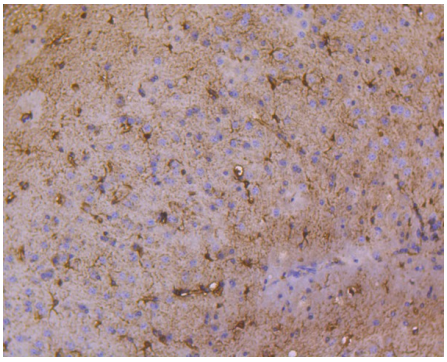

Fig5: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-IKB alpha antibody (ET1603-6) at 1/200 dilution.

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 (ET1603-6) 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.

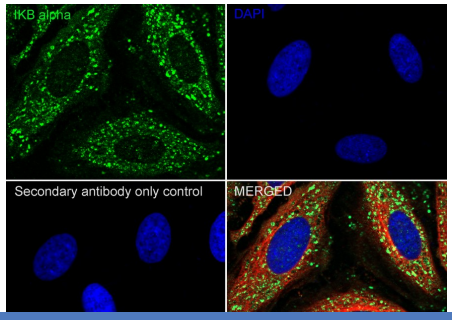

Fig6: Immunocytochemistry analysis of HeLa cells labeling IKB alpha with Rabbit anti-IKB alpha antibody (ET1603-6) at 1/100 dilution.

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-IKB alpha antibody (ET1603-6) 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

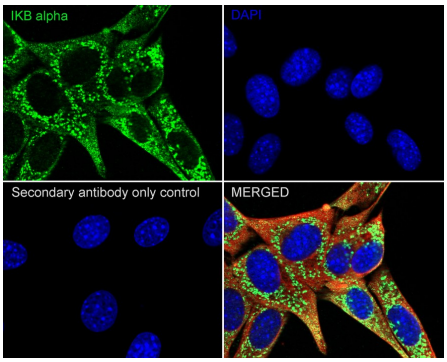

Fig7: Immunocytochemistry analysis of NIH/3T3 cells labeling IKB alpha with Rabbit anti-IKB alpha antibody (ET1603-6) at 1/100 dilution.

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-IKB alpha antibody (ET1603-6) 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.

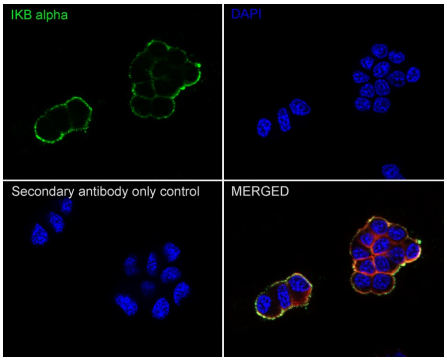

Fig8: Immunocytochemistry analysis of PC-12 cells labeling IKB alpha with Rabbit anti-IKB alpha antibody (ET1603-6) at 1/100 dilution.

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-IKB alpha antibody (ET1603-6) 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.

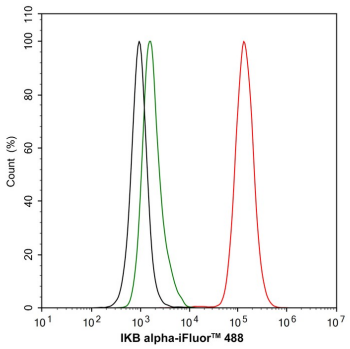

Fig9: Flow cytometric analysis of HeLa cells labeling IKB alpha.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1603-6, 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).

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

Background References

1. Bai X et al. Prostaglandin E2 stimulates 1-integrin expression in hepatocellular carcinoma through the EP1 receptor/PKC/NF- B pathway. Sci Rep 4:6538 (2014).
2. Broderick TL et al. Downregulation in GATA4 and Downstream Structural and Contractile Genes in the db/db Mouse Heart. ISRN Endocrinol 2012:736860 (2012).