Anti-IKK alpha + IKK beta Antibody [SN63-02] ET1611-23

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IP
Molecular Wt:	Predicted band size: 87 kDa
Clone number:	SN63-02

Description: IKK-α is part of the IκB kinase complex that plays an important role in regulating the NF-κB transcription factor. IKK-β is an enzyme that serves as a protein subunit of IκB kinase, which is a component of the cytokine-activated intracellular signaling pathway involved in triggering immune responses. IKK's activity causes activation of a transcription factor known as Nuclear Transcription factor kappa-B or NF-κB. Activated IKK-β phosphorylates a protein called the inhibitor of NF-κB, IκB (IκBα), which binds NF-κB to inhibit its function. IKK-α and IKK-β phosphorylate the IκB proteins, marking them for degradation via ubiquitination and allowing NF-κB transcription factors to go into the nucleus. Once activated, NF-κB transcription factors regulate genes that are implicated in many important cellular processes, including immune response, inflammation, cell death, and cell proliferation.

Immunogen: Recombinant protein within Human IKK beta aa 450-652 / 756.

Positive control: HeLa cell lysate, 293T cell lysate, Daudi cell lysate, A431, NIH/3T3, C6.

Subcellular location: Cytoplasm, Nucleus, Membrane raft.

Database links: SwissProt: O14920 Human | O15111 Human | O88351 Mouse | Q60680 Mouse | Q9QY78 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:100-1:500
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

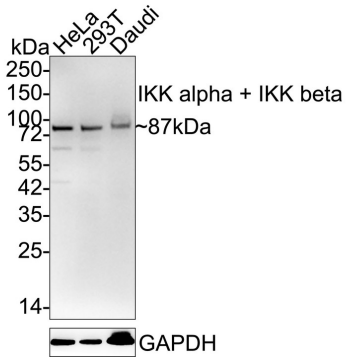

Fig1: Western blot analysis of IKK alpha + IKK beta on different lysates with Rabbit anti-IKK alpha + IKK beta antibody (ET1611-23) at 1/2,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: 293T cell lysate
Lane 3: Daudi cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 87 kDa
Observed band size: 87 kDa

Exposure time: 1 minute 2 seconds; ECL: K1802;

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 (ET1611-23) at 1/2,000 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.

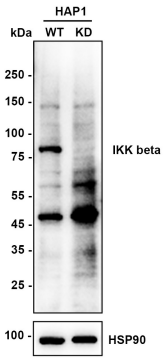

Fig2: Western blot analysis of IKK alpha + IKK beta on different lysates with Rabbit anti-IKK alpha + IKK beta antibody (ET1611-23) at 1/5,000 dilution.

Lane 1: HAP1-parental cell lysate
Lane 2: HAP1-IKK beta KD cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 87 kDa
Observed band size: 87 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 (ET1611-23) at 1/5,000 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.

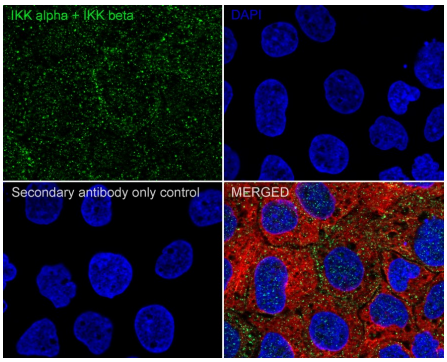

Fig3: Immunocytochemistry analysis of A431 cells labeling IKK alpha + IKK beta with Rabbit anti-IKK alpha + IKK beta antibody (ET1611-23) at 1/500 dilution.

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-IKK alpha + IKK beta antibody (ET1611-23) at 1/500 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.

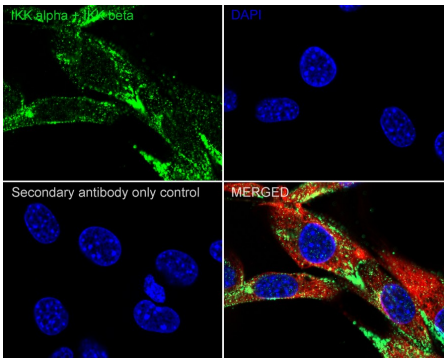

Fig4: Immunocytochemistry analysis of NIH/3T3 cells labeling IKK alpha + IKK beta with Rabbit anti-IKK alpha + IKK beta antibody (ET1611-23) at 1/500 dilution.

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-IKK alpha + IKK beta antibody (ET1611-23) at 1/500 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.

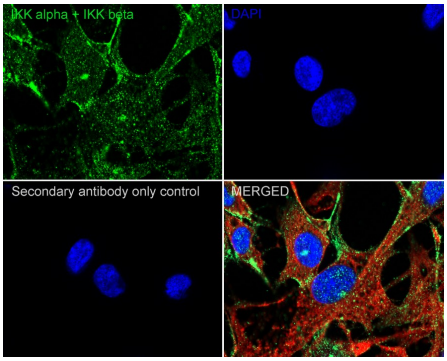

Fig5: Immunocytochemistry analysis of C6 cells labeling IKK alpha + IKK beta with Rabbit anti-IKK alpha + IKK beta antibody (ET1611-23) at 1/250 dilution.

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-IKK alpha + IKK beta antibody (ET1611-23) at 1/250 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.

Background References

1. Hwang, B. et al. 2015. IPO3-mediated Nonclassical Nuclear Import of NF-κB Essential Modulator (NEMO) Drives DNA Damage-dependent NF-κB Activation. The Journal of biological chemistry. 290: 17967-84.
2. Muhammad, K. et al. 2014. NF-κB factors control the induction of NFATc1 in B lymphocytes. European journal of immunology. 44: 3392-402.