Anti-Phospho-IKB alpha (S32) Antibody [ST53-05] - BSA and Azide free

HA750196

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse
Applications:	WB, IF-Cell, IP
Molecular Wt:	Predicted band size: 36 kDa
Clone number:	ST53-05

Description: On the basis of both functional and structural considerations, members of the IκB family of proteins can be divided into four groups. The first of these groups, IκB-α, includes the avian protein pp40 and the mammalian MAD-3, both of which inhibit binding of p50-p65 NFkB complex or Rel protein to their cognate binding sites but do not inhibit the binding of p50 homodimer to κB sites, suggesting that the IκB-α family binds to the p65 subunit of p50-p65 heterocomplex through ankyrin repeats. The second member of the IκB family is represented by a protein designated IκB-β. The third group of IκB proteins is represented by IκB-γ, which is identical in sequence with the C-terminal domain of the p110 precursor of NFkB p50 and is expressed predominantly in lymphoid cells. An additional IκB family member, IκB-ε, has several phosphorylated forms and is primarily found complexed with Rel A and/or c-Rel.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Ser32 of human IKB alpha.

Positive control: HeLa treated with 20ng/mL TNF-α for 5 minutes cell lysate, HeLa treated with 100nM Calyculin A for 30 minutes cell lysate, RAW264.7 treated with 50ng/mL Calyculin A for 45 minutes cell lysate, NIH/3T3 cells were starved for 18 hours then treated with 20 ng/ml TNF alpha for 5 minutes, HeLa.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: P25963 Human | Q9Z1E3 Mouse

Recommended Dilutions:

WB	1:1,000-1:5,000
IF-Cell	1:50-1:200
IP	Use at an assay dependent concentration.

Storage Buffer: PBS (pH7.4).

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

Purity: Protein A affinity purified.

Images

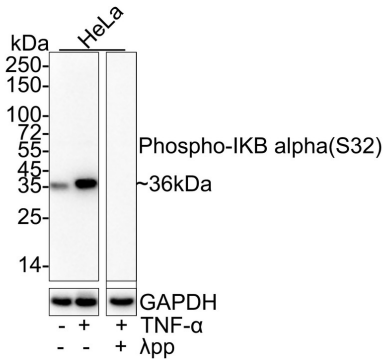

Fig1: Western blot analysis of Phospho-IKB alpha (S32) on different lysates with Rabbit anti-Phospho-IKB alpha (S32) antibody (HA750196) at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with 20ng/mL TNF-α for 5 minutes cell lysate
Lane 3: HeLa treated with 20ng/mL TNF-α for 5 minutes cell lysate, then the membrane treated with λpp for 1 hour

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 1 minute 2 seconds;
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 (HA750196) at 1/1,000 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.

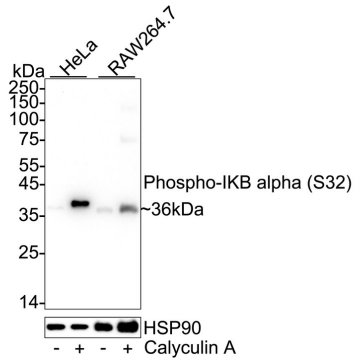

Fig2: Western blot analysis of Phospho-IKB alpha (S32) on different lysates with Rabbit anti-Phospho-IKB alpha (S32) antibody (HA750196) at 1/5,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)
Lane 2: HeLa treated with 100nM Calyculin A for 30 minutes cell lysate (20 µg/Lane)
Lane 3: RAW264.7 cell lysate (20 µg/Lane)
Lane 4: RAW264.7 treated with 50ng/mL Calyculin A for 45 minutes cell lysate (20 µg/Lane)

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

Exposure time: 59 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 (HA750196) 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.

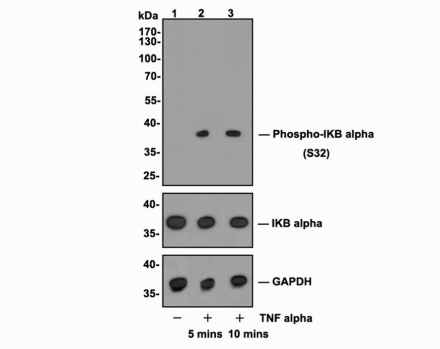

Fig3: Western blot analysis of Phospho-IKB alpha (S32) on NIH-3T3 cell lysates.

Lane 1: NIH/3T3 cells were starved for 18 hours, whole cell lysate, 10 ug/lane.
Lane 2: NIH/3T3 cells were starved for 18 hours, then treated with 20 ng/ml TNF alpha for 5 minutes, whole cell lysates, 10 ug/lane.
Lane 3: NIH/3T3 cells were starved for 18 hours, then treated with 20 ng/ml TNF alpha for 10 minutes, whole cell lysates, 10 ug/lane.

Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody Anti-Phospho-IKB alpha (S32) (HA750196, 1/1,000) , Anti-IKB alpha antibody (ET1603-6, 1/2,000) and Anti-GAPDH antibody (ET1601-4, 1/10,000)was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG H&L (HRP) Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.

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

Exposure time: 1 minute

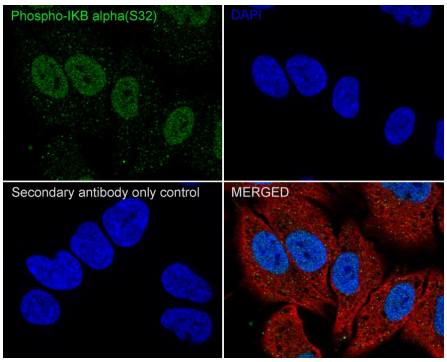

Fig4: Immunocytochemistry analysis of HeLa cells labeling Phospho-IKB alpha (S32) with Rabbit anti-Phospho-IKB alpha (S32) antibody (HA750196) at 1/100 dilution.

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-Phospho-IKB alpha (S32) antibody (HA750196) 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.

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

Background References

1. Liu Y et al. The natural compound magnolol inhibits invasion and exhibits potential in human breast cancer therapy. Sci Rep 3:3098 (2013).
2. Kiefel H et al. EMT-associated up-regulation of L1CAM provides insights into L1CAM-mediated integrin signalling and NF- B activation. Carcinogenesis 33:1919-29 (2012).