# Anti-Phospho-IKB alpha (S32/S36) Antibody [PSH06-76] HA722770

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell
Molecular Wt:	Predicted band size: 36 kDa
Clone number:	PSH06-76
Description:	IκBα (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha; NFKBIA) is one member of a family of cellular proteins that function to inhibit the NF-κB transcription factor. IκBα inhibits NF-κB by masking the nuclear localization signals (NLS) of NF-κB proteins and keeping them sequestered in an inactive state in the cytoplasm. In addition, IκBα blocks the ability of NF-κB transcription factors to bind to DNA, which is required for NF-κB's proper functioning.
lmmunogen:	Synthetic phospho-peptide corresponding to residues surrounding Ser32/Ser36 of human IKB alpha aa 1-50.
Positive control:	HeLa treated with 20ng/mL TNF- $\alpha$ and 100nM Calyculin A for 10 minutes cell lysate, NIH/3T3 treated with 20ng/mL TNF- $\alpha$ and 100nM Calyculin A for 10 minutes cell lysate, C6 treated with 100ng/mL Calyculin A for 1 hours cell lysate, human breast cancer tissue, HeLa cells treated with 20ng/mL TNF- $\alpha$ and 100nM Calyculin A for 10 minutes, NIH/3T3 cells treated with 20ng/mL TNF- $\alpha$ and 100nM Calyculin A for 10 minutes.
Subcellular location:	Cytoplasm, Nucleus.
Database links:	SwissProt: P25963 Human   Q9Z1E3 Mouse   Q63746 Rat
Recommended Dilutions: WB IHC-P IF-Cell	1:5,000 1:200 1:100-1:1,000
Storage Buffer:	PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
Storage Instruction:	Store at +4 $^\circ\!\mathrm{C}$ after thawing. Aliquot store at -20 $^\circ\!\mathrm{C}$ . Avoid repeated freeze / thaw cycles.
Purity:	Protein A affinity purified.

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Orders:0086-571-88062880

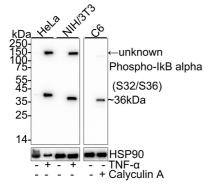
Technical:0086-571-89986345

5 Service mail:support@huabio.cn



Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

#### Images



**Fig1:** Western blot analysis of Phospho-IKB alpha (S32/S36) on different lysates with Rabbit anti-Phospho-IKB alpha (S32/S36) antibody (HA722770) at 1/5,000 dilution.

Lane 1: HeLa cell lysate

Lane 2: HeLa treated with 20ng/mL TNF-α and 100nM Calyculin A for 10 minutes cell lysate Lane 3: NIH/3T3 cell lysate Lane 4: NIH/3T3 treated with 20ng/mL TNF-α and 100nM Calyculin A for 10 minutes cell lysate Lane 5: C6 cell lysate Lane 6: C6 treated with 100ng/mL Calyculin A for 1 hours cell

Lysates/proteins at 20 µg/Lane.

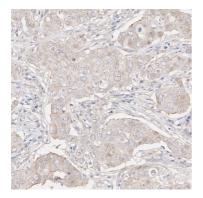
lysate

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

Exposure time: 3 minutes; 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 (HA722770) at 1/5,000 dilution was used in 5% NFDM/TBST at  $4^{\circ}$ C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.



**Fig2:** Immunohistochemical analysis of paraffin-embedded human breast cancer tissue with Rabbit anti-Phospho-IKB alpha (S32/S36) antibody (HA722770) 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<sub>2</sub>O and PBS, and then probed with the primary antibody (HA722770) 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.

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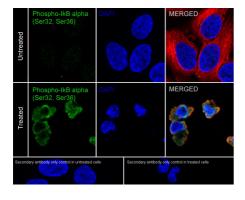
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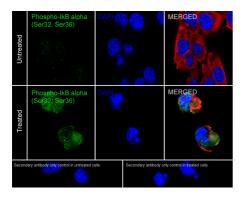
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**Fig3:** Immunocytochemistry analysis of HeLa cells treated with 20ng/mL TNF- $\alpha$  and 100nM Calyculin A for 10 minutes labeling Phospho-IKB alpha (S32/S36) with Rabbit anti-Phospho-IKB alpha (S32/S36) antibody (HA722770) at 1/1,000 dilution.

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/S36) antibody (HA722770) at 1/1,000 dilution in 1% BSA in PBST overnight at 4  $^{\circ}$ C. Goat Anti-Rabbit IgG H&L (iFluor <sup>TM</sup> 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  $+4^{\circ}$ C. Goat Anti-Mouse IgG H&L (iFluor <sup>TM</sup> 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.



**Fig4:** Immunocytochemistry analysis of NIH/3T3 cells treated with 20ng/mL TNF- $\alpha$  and 100nM Calyculin A for 10 minutes labeling Phospho-IKB alpha (S32/S36) with Rabbit anti-Phospho-IKB alpha (S32/S36) antibody (HA722770) 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/S36) antibody (HA722770) at 1/100 dilution in 1% BSA in PBST overnight at 4  $^{\circ}$ C. Goat Anti-Rabbit IgG H&L (iFluor M 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  $+4^{\circ}$ C. Goat Anti-Mouse IgG H&L (iFluor 1594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

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

#### **Background References**

- 1. Chen WJ et al. PNU-74654 Suppresses TNFR1/IKB Alpha/p65 Signaling and Induces Cell Death in Testicular Cancer. Curr Issues Mol Biol. 2022 Jan
- Liang WJ et al. HMGB1 upregulates NF-kB by inhibiting IKB-alpha and associates with diabetic retinopathy. Life Sci. 2020 Jan

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