abcam

Product datasheet

Anti-IKB alpha antibody [E130] ab32518

KO VALIDATED Recombinant RabMAb®

★★★★☆ **31 Abreviews**   **385 References**   18 Images

Overview

Product name	Anti-IKB alpha antibody [E130]
Description	Rabbit monoclonal [E130] to IKB alpha
Host species	Rabbit
Specificity	This antibody detects both the phosphorylated and non-phosphorylated form of the serine 32 region of IKB alpha.
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC/IF, WB, IHC-P, IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
	Predicted to work with: Hamster, Cow, Pig
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Wild type HAP1 whole cell lysate. HeLa whole cell lysate. PC-12, SH-SY5Y, HepG2, K562, NIH/3T3 and RAW 264.7 cell lysate. Human fetal liver lysate. IHC-P: Human prostate carcinoma and stomach tissue. Rat and mouse kidney tissue. ICC/IF: HeLa and RAW 264.7 cells. IP: IKB alpha IP in HeLa cell lysate. PC-12 whole cell lysate. Flow Cyt (intra): HeLa cells.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA

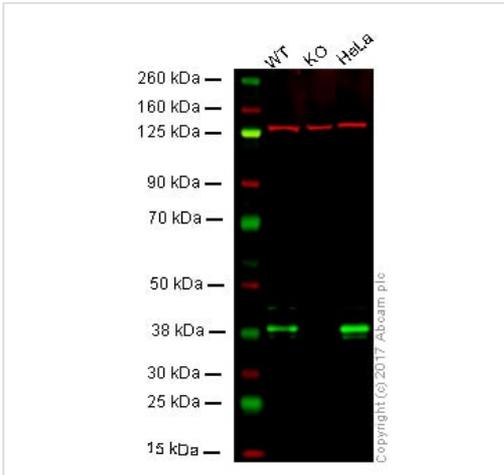|		
Purity	Protein A purified	
Clonality	Monoclonal	
Clone number	E130	
Isotype	IgG	

Applications

The Abpromise guarantee   Our **Abpromise guarantee** covers the use of ab32518 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/20.
ICC/IF		1/50.
WB	★★★★★ (27)	1/1000 - 1/100000. Detects a band of approximately 35 kDa (predicted molecular weight: 36 kDa).
IHC-P	★★★★☆ (1)	1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
IP	★★★★★ (2)	1/20.

Target

Function	Inhibits the activity of dimeric NF-kappa-B/REL complexes by trapping REL dimers in the cytoplasm through masking of their nuclear localization signals. On cellular stimulation by immune and proinflammatory responses, becomes phosphorylated promoting ubiquitination and degradation, enabling the dimeric RELA to translocate to the nucleus and activate transcription.
Involvement in disease	Ectodermal dysplasia, anhidrotic, with T-cell immunodeficiency autosomal dominant
Sequence similarities	Belongs to the NF-kappa-B inhibitor family. Contains 5 ANK repeats.
Post-translational modifications	Phosphorylated; disables inhibition of NF-kappa-B DNA-binding activity. Phosphorylation at positions 32 and 36 is prerequisite to recognition by UBE2D3 leading to polyubiquitination and subsequent degradation. Sumoylated; sumoylation requires the presence of the nuclear import signal. Sumoylation blocks ubiquitination and proteasome-mediated degradation of the protein thereby increasing the protein stability. Monoubiquitinated at Lys-21 and/or Lys-22 by UBE2D3. Ubiquitin chain elongation is then performed by CDC34 in cooperation with the SCF(FBXW11) E3 ligase complex, building ubiquitin chains from the UBE2D3-primed NFKBIA-linked ubiquitin. The resulting polyubiquitination leads to protein degradation. Also ubiquitinated by SCF(BTRC) following stimulus-dependent phosphorylation at Ser-32 and Ser-36. Deubiquitinated by porcine reproductive and respiratory syndrome virus Nsp2 protein, which thereby interferes with NFKBIA degradation and impairs subsequent NF-kappa-B activation.
Cellular localization	Cytoplasm. Nucleus. Shuttles between the nucleus and the cytoplasm by a nuclear localization

signal (NLS) and a CRM1-dependent nuclear export.

Images

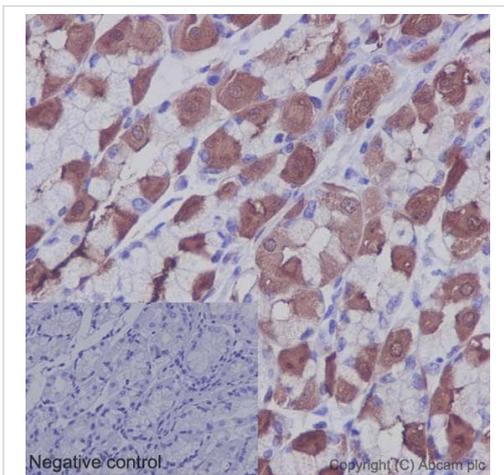Western blot - Anti-IKB alpha antibody [E130]
(ab32518)

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)

Lane 2: IKB alpha knockout HAP1 whole cell lysate (20 µg)

Lane 3: HeLa whole cell lysate (20 µg)

Lanes 1 - 3: Merged signal (red and green). Green - ab32518 observed at 38 kDa. Red - loading control, **ab18058**, observed at 130 kDa.

ab32518 was shown to specifically react with IKB alpha in wild-type HAP1 cells. No band was observed when IKB alpha knockout samples were tested. Wild-type and IKB alpha knockout samples were subjected to SDS-PAGE. ab32518 and **ab18058** (Mouse anti Vinculin loading control) were incubated overnight at 4°C at 1/10,000 dilution and 1/20,000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1/20,000 dilution for 1 hour at room temperature before imaging.


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IKB alpha antibody [E130] (ab32518)

Immunohistochemical staining of paraffin embedded human stomach with purified ab32518 at a working dilution of 1 in 100.

The secondary antibody used is a HRP polymer for rabbit IgG. The sample is counterstained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0.

PBS was used instead of the primary antibody as the **negative control**, and is shown in the inset.
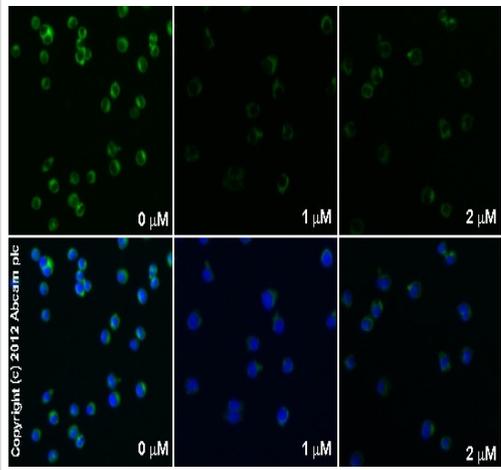
Immunocytochemistry/ Immunofluorescence - Anti-IKB alpha antibody [E130] (ab32518)

Unpurified ab32518 staining IKB alpha in RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) cells treated with FK506 (**ab120223**), by ICC/IF. Decrease in IkBα/&beta expression correlates with increased concentration of FK506, as described in literature.

The cells were incubated at 37°C for 3h in media containing different concentrations of **ab120223** (FK506) in DMSO, fixed with 100% methanol for 5 minutes at -20°C and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature.

Staining of the treated cells with ab32518 (1/100 dilution) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight® 488 goat anti-rabbit polyclonal antibody (**ab96899**) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.
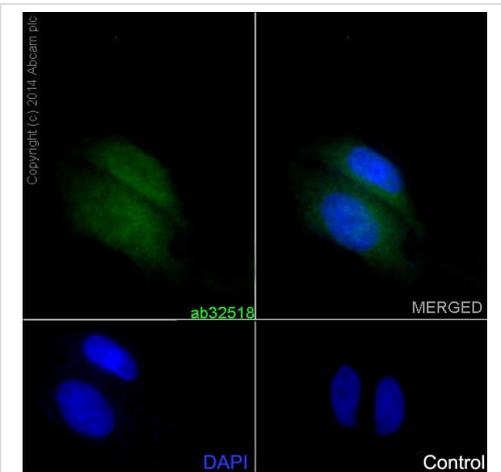

Immunocytochemistry/ Immunofluorescence - Anti-IKB alpha antibody [E130] (ab32518)

Immunofluorescence staining of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells with purified ab32518 at a working dilution of 1 in 50, counterstained with DAPI.

The secondary antibody was **ab150077**, Alexa Fluor® 488 goat anti rabbit, used at a dilution of 1 in 500. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100.

The **negative control** is shown in bottom right hand panel - for the negative control, purified ab32518 was used at a dilution of 1/50 followed by **ab150120**, Alexa Fluor® 594 goat anti-mouse antibody at a dilution of 1/500.
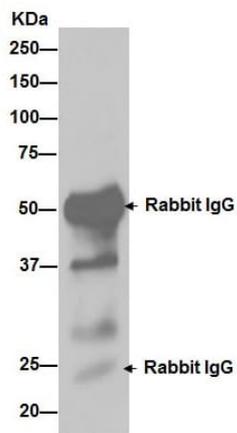
Immunoprecipitation - Anti-IKB alpha antibody [E130] (ab32518)

ab32518 (purified) at 1/20 immunoprecipitating IKB alpha in HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate (**Lane 1**). For western blotting a HRP-conjugated goat anti-rabbit IgG was used as the secondary antibody (1/1000).

Blocking/Dilution buffer and concentration: 5% NFDM/TBST.
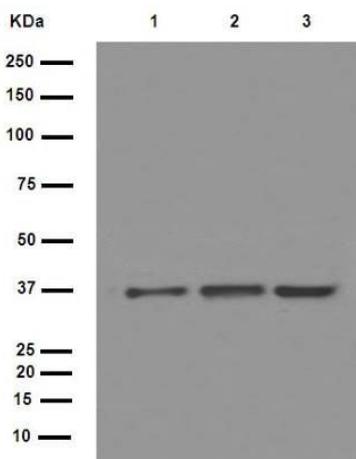
Western blot - Anti-IKB alpha antibody [E130] (ab32518)

All lanes : Anti-IKB alpha antibody [E130] (ab32518) at 1/10000 dilution (purified)

Lane 1 : PC-12 (Rat adrenal gland pheochromocytoma cell line) cell lysate

Lane 2 : NIH/3T3 (Mouse embryo fibroblast cell line) cell lysate

Lane 3 : RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) cell lysate
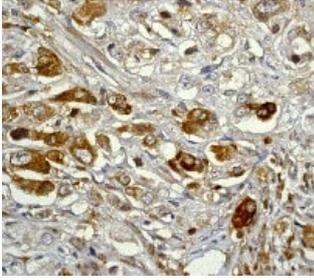
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : HRP goat anti-rabbit IgG (H+L) at 1/1000 dilution
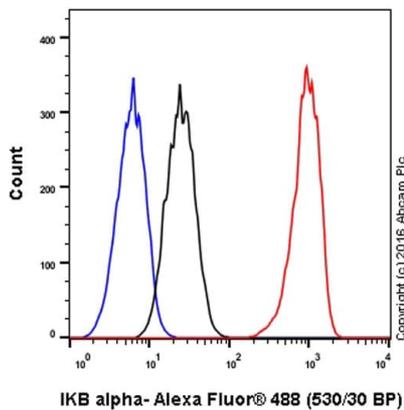
Predicted band size: 36 kDa
Observed band size: 35 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Immunohistochemical analysis of paraffin-embedded human prostate carcinoma using unpurified ab32518 at 1/50 dilution.

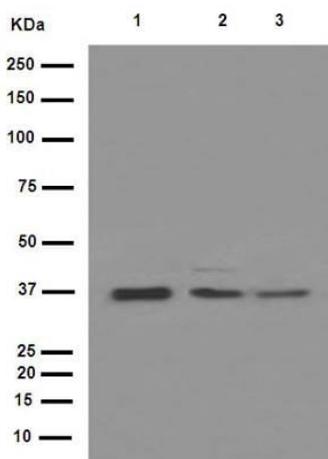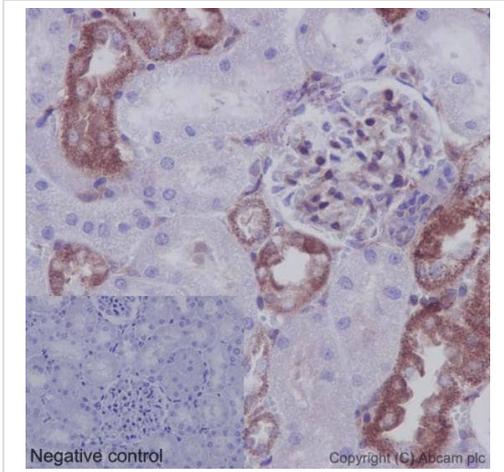Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IKB alpha antibody [E130] (ab32518)

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling IKB alpha with purified ab32518 at 1/20 dilution (10µg/mL) (**red**).

Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. A Goat anti rabbit IgG (Alexa Fluorr® 488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (**Black**) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (**Blue**) were used as the unlabeled control.

Flow Cytometry (Intracellular) - Anti-IKB alpha antibody [E130] (ab32518)

All lanes : Anti-IKB alpha antibody [E130] (ab32518) at 1/10000 dilution (purified)

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate
Lane 2 : K562 Human chronic myelogenous leukemia cell line from bone marrow) cell lysate
Lane 3 : HepG2 (Human liver hepatocellular carcinoma cell line) cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : HRP goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 36 kDa

Western blot - Anti-IKB alpha antibody [E130] (ab32518)

Observed band size: 35 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Immunohistochemical staining of paraffin embedded rat kidney with purified ab32518 at a working dilution of 1 in 100.

The secondary antibody used is a HRP polymer for rabbit IgG. The sample is counterstained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0.

PBS was used instead of the primary antibody as the **negative control**, and is shown in the inset.
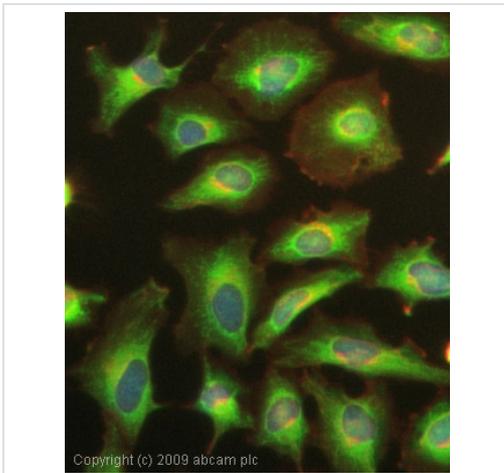
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IKB alpha antibody [E130] (ab32518)

ICC/IF image of unpurified ab32518 stained HeLa (Human epithelial cell line from cervix adenocarcinoma) cells.

The cells were fixed in 100% methanol (5 min) and then incubated in 1% BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilize the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab32518, 1/1000 dilution) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h.

DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43 µM.
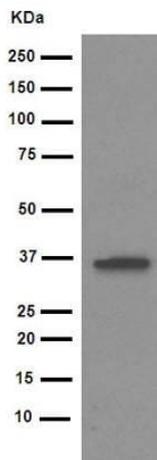
Immunocytochemistry/ Immunofluorescence - Anti-IKB alpha antibody [E130] (ab32518)

Western blot - Anti-IKB alpha antibody [E130] (ab32518)

Anti-IKB alpha antibody [E130] (ab32518) at 1/1000 dilution (purified) + Human fetal liver at 10 µg
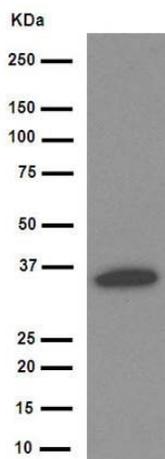
Secondary

HRP anti-rabbit IgG, specific to the non reduced form of IgG at 1/1000 dilution

Predicted band size: 36 kDa
Observed band size: 35 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Western blot - Anti-IKB alpha antibody [E130] (ab32518)

Anti-IKB alpha antibody [E130] (ab32518) at 1/1000 dilution (purified) + SH-SY5Y cell lysate at 10 µg
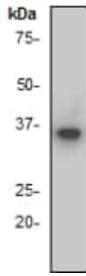
Secondary

HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 36 kDa
Observed band size: 35 kDa

Blocking buffer: 5% NFDM/TBST
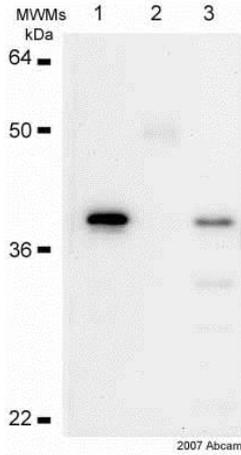
Dilution buffer: 5% NFDM/TBST

Anti-IKB alpha antibody [E130] (ab32518) at 1/10000 dilution (unpurified) + HeLa cell lysate

Predicted band size: 36 kDa
Observed band size: 35 kDa
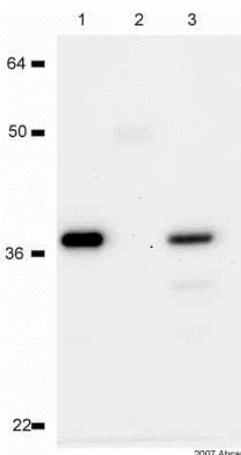
Western blot - Anti-IKB alpha antibody [E130] (ab32518)

Unpurified ab32518 used to immunoprecipitate IKB alpha from HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate. The antibody was further used to Western blot the protein.

Lane 1: IKB alpha IP

Lane 2: Control immunoprecipitate

Lane 3: Input (20%)

Immunoprecipitation - Anti-IKB alpha antibody [E130] (ab32518)

This image is courtesy of an anonymous Abreview

Unpurified ab32518 used to immunoprecipitate IKB alpha from PC-12 (Rat adrenal gland pheochromocytoma cell line) whole cell lysate. The antibody was further used to Western blot the protein.

Lane 1: IKB alpha IP

Lane 2: Control immunoprecipitate

Lane 3: Input (20%)

Immunoprecipitation - Anti-IKB alpha antibody [E130] (ab32518)

This image is courtesy of an anonymous Abreview

Unpurified ab32518 at 1/100 staining mouse kidney tissue sections by IHC-P.

The tissue was paraformaldehyde fixed and a heat mediated antigen retrieval step in citrate buffer was performed before the tissue was blocked and incubated with the antibody for 1 hour. An HRP conjugated goat anti-rabbit antibody was used as the secondary.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IKB alpha antibody [E130] (ab32518)

This image is courtesy of an anonymous Abreview

Why choose a recombinant antibody?

Research with confidence
Consistent and reproducible results

Long-term and scalable supply
Recombinant technology

Success from the first experiment
Confirmed specificity

Ethical standards compliant
Animal-free production

Anti-IKB alpha antibody [E130] (ab32518)

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