abcam

Product datasheet

Anti-IKB alpha antibody [E130] - BSA and Azide free ab215972

KO VALIDATED Recombinant RabMAb

25 References 13 Images

Overview

Product name	Anti-IKB alpha antibody [E130] - BSA and Azide free
Description	Rabbit monoclonal [E130] to IKB alpha - BSA and Azide free
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), WB, IP, ICC/IF, IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Cow, Pig
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	Hela cell lysate and human prostate carcinoma tissue.
General notes	ab215972 is the carrier-free version of **ab32518**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	E130
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab215972 in the following tested applications.

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

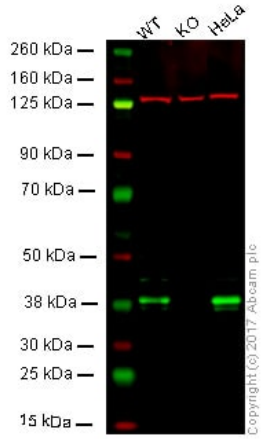
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 35 kDa (predicted molecular weight: 36 kDa).
IP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

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	Phosphorylated; disables inhibition of NF-kappa-B DNA-binding activity. Phosphorylation at

modifications	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



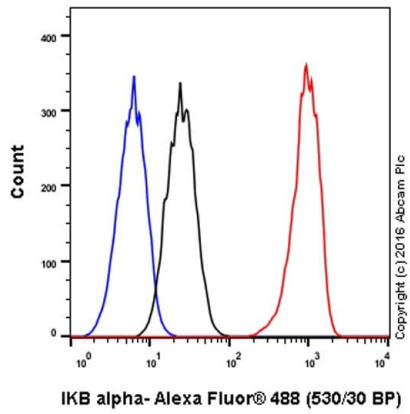
Western blot - Anti-IKB alpha antibody [E130] - BSA and Azide free (ab215972)

This WB data was generated using the same anti-IKB alpha antibody clone, E130, in a different buffer formulation (cat# **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.

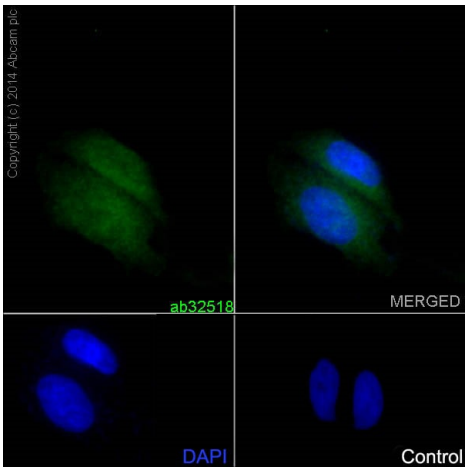
ab32518 was shown to specifically react with IKB alpha in wild-type HAP1 cells. No band was observed when IKB alpha knockout samples were tested. Wild-type and IKB alpha knockout samples were subjected to SDS-PAGE. Ab32518 and **ab18058** (Mouse anti Vinculin loading control) were incubated overnight 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.



Flow Cytometry (Intracellular) - Anti-IKB alpha antibody [E130] - BSA and Azide free (ab215972)

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling IKB alpha with purified **ab32518** at 1/20 dilution (10ug/mL) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 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.

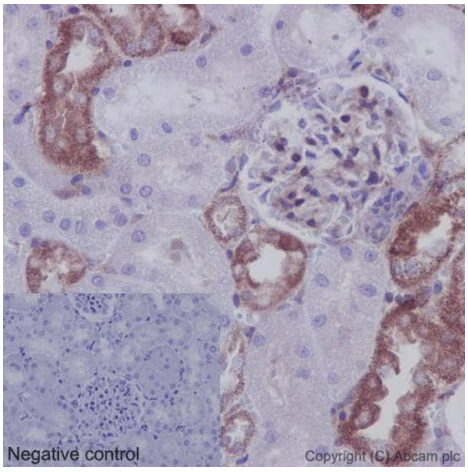
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32518**).



Immunocytochemistry/ Immunofluorescence - Anti-IKB alpha antibody [E130] - BSA and Azide free (ab215972)

Immunofluorescence staining of HeLa cells with purified **ab32518** at a working dilution of 1 in 50, counter-stained 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.

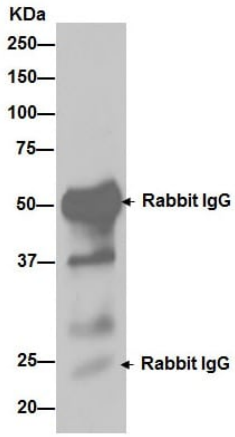
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32518**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IKB alpha antibody [E130] - BSA and Azide free (ab215972)

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 counter-stained 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32518**).



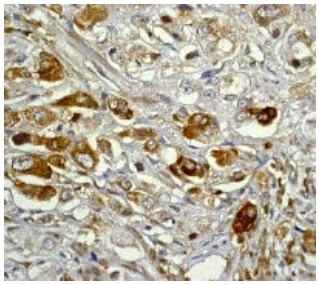
Immunoprecipitation - Anti-IKB alpha antibody [E130] - BSA and Azide free (ab215972)

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

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

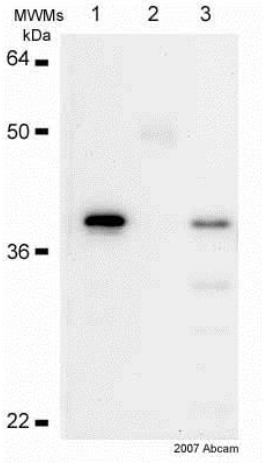
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32518**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IKB alpha antibody [E130] - BSA and Azide free (ab215972)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32518**).



Immunoprecipitation - Anti-IKB alpha antibody [E130] - BSA and Azide free (ab215972)

This image is courtesy of an anonymous Abreview.

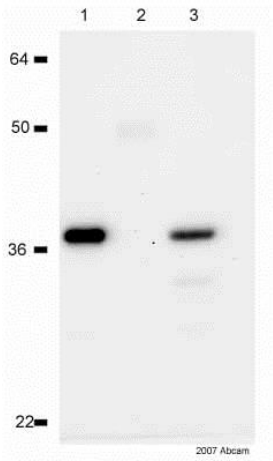
Unpurified **ab32518** used to immunoprecipitate IKB alpha from human HeLa whole cell lysate. The antibody was further used to Western blot the protein.

Lane 1 IKB alpha IP

Lane 2 Control immunoprecipitate

Lane 3 Input (20%)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32518**).



Immunoprecipitation - Anti-IKB alpha antibody [E130] - BSA and Azide free (ab215972)

This image is courtesy of an anonymous Abreview.

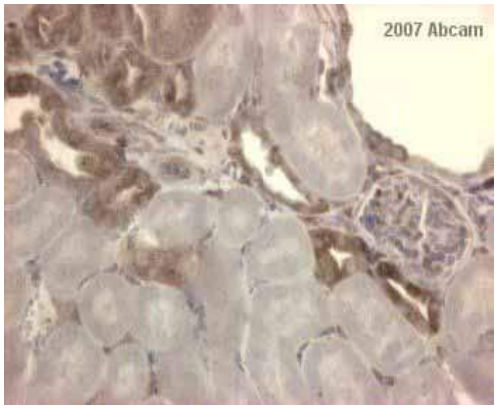
Unpurified **ab32518** used to immunoprecipitate IKB alpha from rat PC12 whole cell lysate. The antibody was further used to Western blot the protein.

Lane 1 IKB alpha IP

Lane 2 Control immunoprecipitate

Lane 3 Input (20%)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32518**).

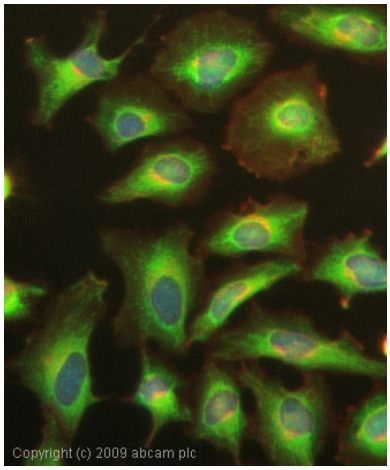


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IKB alpha antibody [E130] - BSA and Azide free (ab215972)

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.

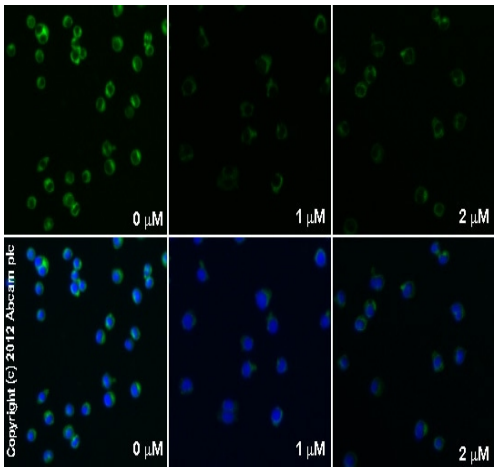
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32518**).



Immunocytochemistry/ Immunofluorescence - Anti-IKB alpha antibody [E130] - BSA and Azide free (ab215972)

ICC/IF image of unpurified **ab32518** stained HeLa cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise 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 1.43µM.

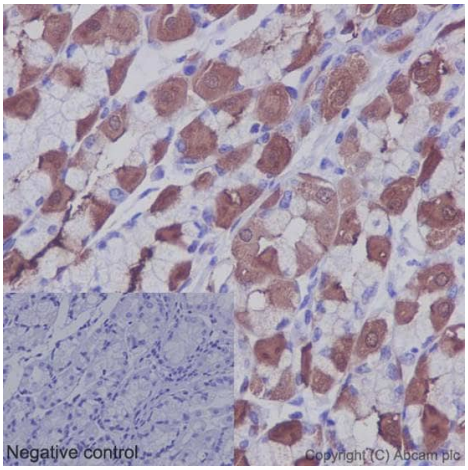
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32518**).



Immunocytochemistry/ Immunofluorescence - Anti-IKB alpha antibody [E130] - BSA and Azide free (ab215972)

Unpurified **ab32518** staining IkBα/&beta in RAW 264.7 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.

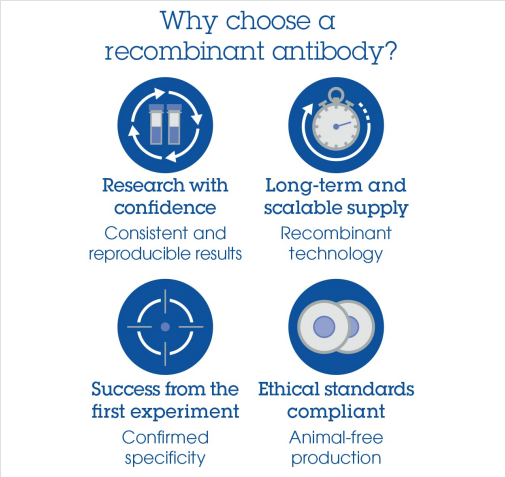
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32518**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IKB alpha antibody [E130] - BSA and Azide free (ab215972)

This IHC data was generated using the same anti-IKB alpha antibody clone, E130, in a different buffer formulation (cat# **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 counter-stained 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.



Anti-IKB alpha antibody [E130] - BSA and Azide free

(ab215972)

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