abcam

ab32518

Anti-IKB alpha antibody [E130]

Anti-IKB alpha antibody [E130] (ab32518) is a rabbit monoclonal antibody detecting IKB alpha in **Western Blot, Flow Cytometry (Intra), IP, IHC-P, ICC/IF**. Suitable for **Human, Mouse, Rat**.

- KO validated for confirmed specificity
- Biophysical QC for unrivalled batch-batch consistency
- Over 440 publications
- Trusted since 2006

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	E130
Purification technique	Affinity purification Protein A
Specificity	This antibody detects both the phosphorylated and non-phosphorylated form of the serine 32 region of IKB alpha.
Concentration	0.119 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Rat
Dilution info	1/100
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Species	Human
Dilution info	1/100
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Hamster, Cow, Pig
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	1/20
Notes	-

Expected

Species	Rat, Mouse
Dilution info	Use at an assay dependent concentration.

Notes	-

Predicted

Species	Hamster, Cow, Pig
Dilution info	-
Notes	-

WB

Tested

Species	Rat
Dilution info	1/1000 - 1/100000
Notes	-

Species	Human
Dilution info	1/1000 - 1/100000
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Hamster, Cow, Pig
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Mouse

Dilution info	1/50
Notes	-

Species	Human
Dilution info	1/50
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Hamster, Cow, Pig
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/20
Notes	-

Expected

Species	Rat, Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Hamster, Cow, Pig

Dilution info	-
Notes	-

Target data

See full target information NFKBIA

Function	Inhibits the activity of dimeric NF-kappa-B/REL complexes by trapping REL (RELA/p65 and NFKB1/p50) dimers in the cytoplasm by masking their nuclear localization signals (PubMed:1493333, PubMed:36651806, PubMed:7479976). On cellular stimulation by immune and pro-inflammatory responses, becomes phosphorylated promoting ubiquitination and degradation, enabling the dimeric RELA to translocate to the nucleus and activate transcription (PubMed:7479976, PubMed:7628694, PubMed:7796813, PubMed:7878466).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Product Specifications

Anti-IKB alpha antibody [E130] (ab32518) was developed by Abcam using patented rabbit monoclonal antibody technology and is validated for use in Flow Cyt (Intra), ICC/IF, IHC-P, IP, WB in human, mouse, rat samples.
Anti-IKB alpha antibody [E130] (ab32518) specifically detects IKB alpha (UniProt ID: P25963; Molecular weight: 36kDa) and is sold in a convenient trial size to enable initial testing (20 µL) and larger sizes for subsequent scaling up experiments (100 µL and 1 mL).

Quality and Validation

Abcam's high quality manufacturing and validation processes ensure Anti-IKB alpha antibody [E130] (ab32518) has high sensitivity and specificity alongside high lot-to-lot consistency and reproducibility.

The specificity of Anti-IKB alpha antibody [E130] (ab32518) has been confirmed by testing in knockout samples.
Anti-IKB alpha antibody [E130] (ab32518) has been cited over 440 times in peer reviewed journals and is trusted by the scientific community.
Anti-IKB alpha antibody [E130] (ab32518) has 31 independent reviews from customers.

Related Products

Conjugation-ready, carrier free format available for antibody clone E130 - ab215972.
Antibody clone E130 is also available pre-conjugated to a variety of labels for your convenience - Alexa Fluor® 488, HRP, Alexa Fluor® 647, Alexa Fluor® 594, Alexa Fluor® 555, APC, PE (ab202644, ab202646, ab202647, ab206346, ab206578, ab224948, ab224949).

Patented technology

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?

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 batch production

For more information, read more on recombinant antibodies.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

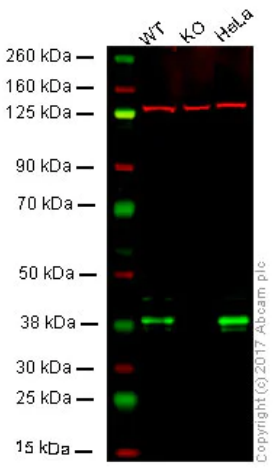
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

20 product images



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

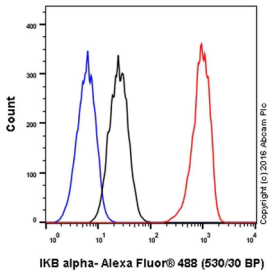
IKB alpha Western blot staining using rabbit Anti-IKB alpha antibody

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.

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

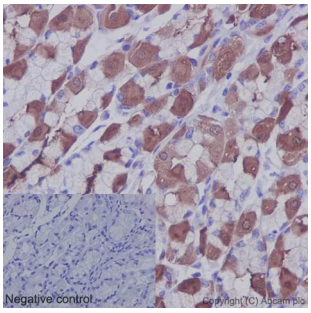
Predicted band size: 35 kDa



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

IKB alpha Flow Cytometry (Intracellular) staining using rabbit Anti-IKB alpha antibody

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.

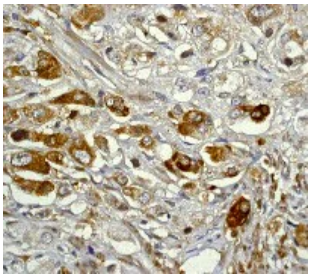


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IKB alpha antibody [E130] (ab32518)

IKB alpha Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-IKB alpha antibody

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.

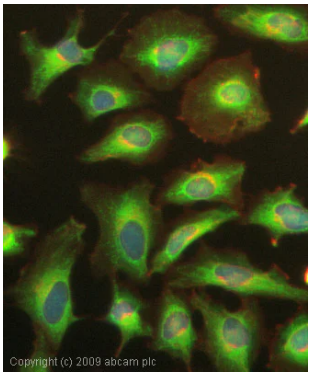
PBS was used instead of the primary antibody as the **negative control**, and is shown in the inset.



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

IKB alpha Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-IKB alpha antibody

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

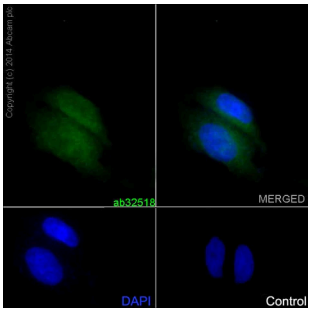


Immunocytochemistry/ Immunofluorescence - Anti-IKB alpha antibody [E130] (ab32518)

IKB alpha Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-IKB alpha antibody

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.

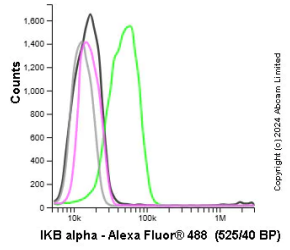


Immunocytochemistry/ Immunofluorescence - Anti-IKB alpha antibody [E130] (ab32518)

IKB alpha Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-IKB alpha antibody

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.



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

IKB alpha Flow Cytometry (Intracellular) staining of HAP1 cells using rabbit Anti-IKB alpha antibody

Flow cytometry overlay histogram showing wild-type HAP1 (green line) and HAP1-NFKBIA knockout cells stained with ab32518 (magenta line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein

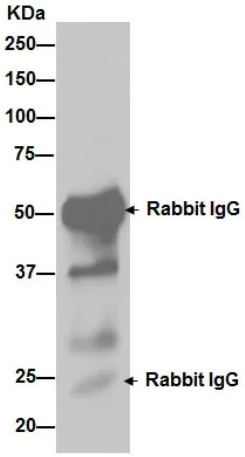
interaction followed by the antibody (ab32518) (1x 10^6in 100µl at 0.04 µg/ml (1/12500)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control was used at the same concentration and conditions as the primary antibody (wild-type HAP1 - black line, HAP1-NFKBIA knockout - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

This antibody gave a positive signal in HAP1 WT Fixed with 80% methanol (5 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



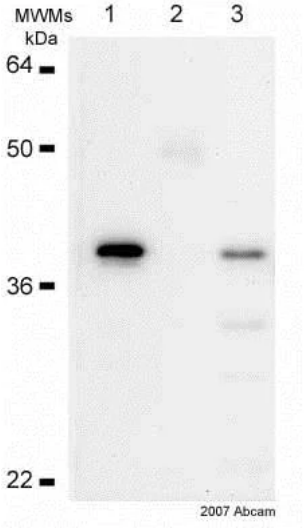
Immunoprecipitation - Anti-IKB alpha antibody [E130] (ab32518)

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.

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

Predicted band size: 35 kDa

Observed band size: 35 kDa



This image is courtesy of an anonymous customer review.

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

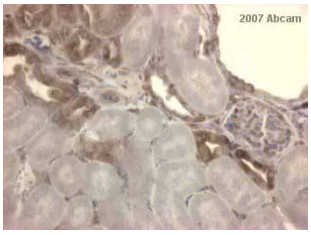
Unpurified ab32518 used to immunoprecipitate IKB alpha from HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate. The antibody was further used to Western blot the protein.
Lane 1: IKB alpha IP

Lane 2: Control immunoprecipitate

Lane 3: Input (20%)

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

Predicted band size: 35 kDa

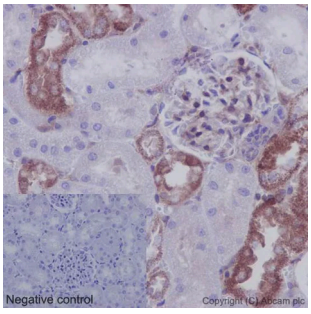


This image is courtesy of an anonymous customer review.

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

IKB alpha Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-IKB alpha antibody

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.

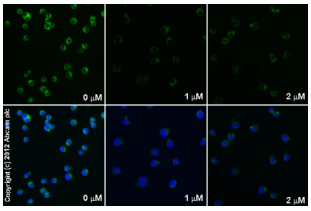


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IKB alpha antibody [E130] (ab32518)

IKB alpha Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-IKB alpha antibody

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.

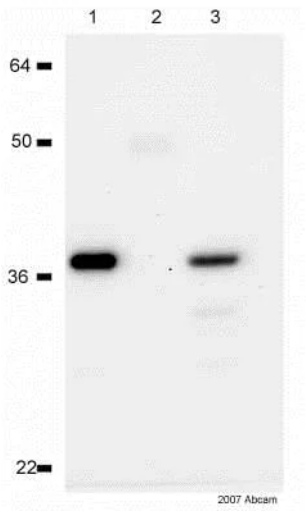
PBS was used instead of the primary antibody as the **negative control**, and is shown in the inset.



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

IKB alpha Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-IKB alpha antibody

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/β 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 image is courtesy of an anonymous customer review.

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

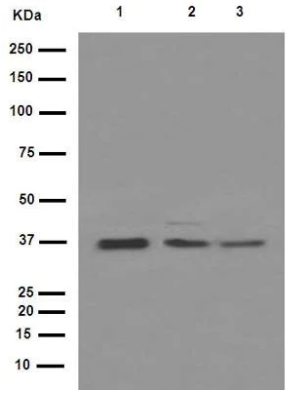
Unpurified ab32518 used to immunoprecipitate IKB alpha from PC-12 (Rat adrenal gland pheochromocytoma cell line) whole cell lysate. The antibody was further used to Western blot the protein.
Lane 1: IKB alpha IP

Lane 2: Control immunoprecipitate

Lane 3: Input (20%)

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

Predicted band size: 35 kDa



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

IKB alpha Western blot staining using rabbit Anti-IKB alpha antibody

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-IKB alpha antibody [E130] (ab32518) at 1/10000 dilution

Lane 1:
HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate at 20 µg

Lane 2:
K562 Human chronic myelogenous leukemia cell line from bone marrow) cell lysate at 20 µg

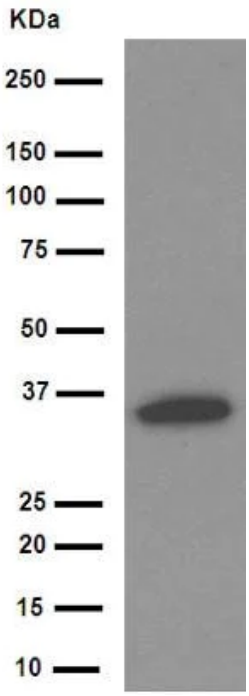
Lane 3:
HepG2 (Human liver hepatocellular carcinoma cell line) cell lysate at 20 µg

Secondary

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

Predicted band size: 35 kDa

Observed band size: 35 kDa



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

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

All lanes:
Western blot - Anti-IKB alpha antibody [E130] (ab32518) at 1/1000 dilution

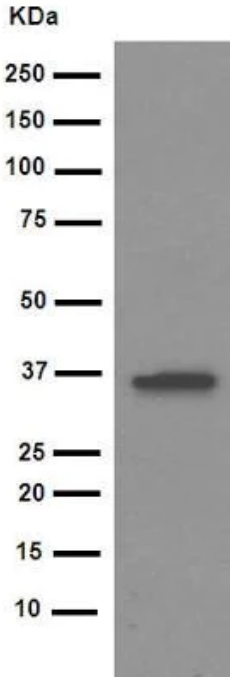
All lanes:
SH-SY5Y cell lysate at 10 µg

Secondary

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

Predicted band size: 35 kDa

Observed band size: 35 kDa



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

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-IKB alpha antibody [E130] (ab32518) at 1/1000 dilution

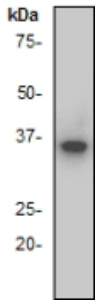
All lanes:
Human fetal liver at 10 µg

Secondary

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

Predicted band size: 35 kDa

Observed band size: 35 kDa



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

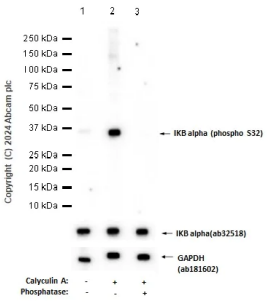
IKB alpha Western blot staining of HeLa cell lysate using rabbit Anti-IKB alpha antibody

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

All lanes:
HeLa cell lysate

Predicted band size: 35 kDa

Observed band size: 35 kDa



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

IKB alpha Western blot staining using rabbit Anti-IKB alpha antibody

Blocking/Diluting buffer and concentration: 5% NFDM/TBST.
All lanes:
Western blot - Anti-IKB alpha (phospho S32) antibody [EPR3148] (ab92700) at 1/1000 dilution

Lane 1:
Untreated C6 (Rat glial tumor glial cell) whole cell lysate at 20 µg

Lane 2:
C6 (Rat glial tumor glial cell) treated with 100 ng/mL Calyculin A for 30 minutes whole cell lysate at 20 µg

Lane 3:
C6 (Rat glial tumor glial cell) treated with 100 ng/mL Calyculin A for 30 minutes whole cell lysate. Then the membrane was incubated with alkaline phosphatase at 20 µg

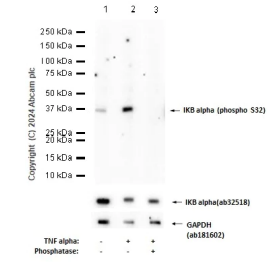
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 35 kDa

Observed band size: 36 kDa

Exposure time: 20s



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

IKB alpha Western blot staining using rabbit Anti-IKB alpha antibody

Blocking/Diluting buffer and concentration: 5% NFDM/TBST.
All lanes:
Western blot - Anti-IKB alpha (phospho S32) antibody [EPR3148] (ab92700) at 1/1000 dilution

Lane 1:
Untreated Raw264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 20 µg

Lane 2:
Raw264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) treated with 50 ng/mL TNF-a and 300 ng/ml BFA for 24 hours whole cell lysate at 20 µg

Lane 3:
Raw264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) treated with 50 ng/mL TNF-a and 300 ng/ml BFA for 24 hours whole cell lysate.Then the membrane was incubated with alkaline phosphatase at 20 µg

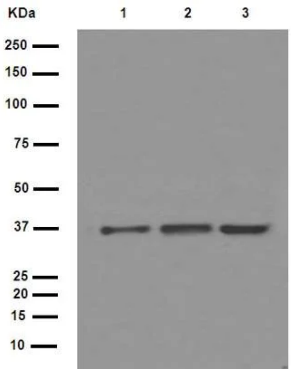
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 35 kDa

Observed band size: 36 kDa

Exposure time: 180s



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

IKB alpha Western blot staining using rabbit Anti-IKB alpha antibody

Blocking/Dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-IKB alpha antibody [E130] (ab32518) at 1/10000 dilution

Lane 1:
PC-12 (Rat adrenal gland pheochromocytoma cell line) cell lysate at 20 µg

Lane 2:
NIH/3T3 (Mouse embryo fibroblast cell line) cell lysate at 20 µg

Lane 3:
RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) cell lysate at 20 µg

Secondary

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

Predicted band size: 35 kDa

Observed band size: 35 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.