abcam

Product datasheet

Anti-IKB alpha (phospho S32) antibody [EPR3148] - BSA and Azide free ab239920

Recombinant RabMAb

7 Images

Overview

Product name	Anti-IKB alpha (phospho S32) antibody [EPR3148] - BSA and Azide free
Description	Rabbit monoclonal [EPR3148] to IKB alpha (phospho S32) - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** IP, Dot blot, WB
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: TNF-a treated HeLa and TNF-a treated MCF7 whole cell lysates, Raw264.7 treated with TNF-a and BFA whole cell lysate, C6 treated with Calyculin A whole cell lysate; IP: TNF-a treated HeLa whole cell lysate.
General notes	ab239920 is the carrier-free version of **ab92700**. 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**.

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR3148
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab239920 in the following tested applications.

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

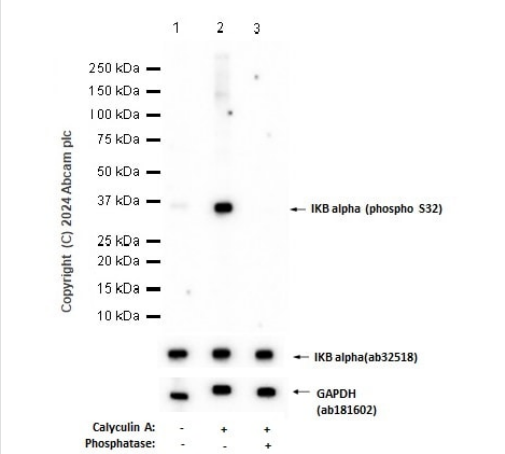
Application	Abreviews	Notes
IP		Use at an assay dependent concentration.
Dot blot		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 36 kDa.

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 (phospho S32) antibody [EPR3148] - BSA and Azide free (ab239920)

All lanes : Anti-IKB alpha (phospho S32) antibody [EPR3148] (**ab92700**) at 1/1000 dilution

Lane 1 : Untreated C6 (Rat glial tumor glial cell) whole cell lysate
Lane 2 : C6 (Rat glial tumor glial cell) treated with 100 ng/mL Calyculin A for 30 minutes whole cell lysate
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

Lysates/proteins at 20 µg per lane.

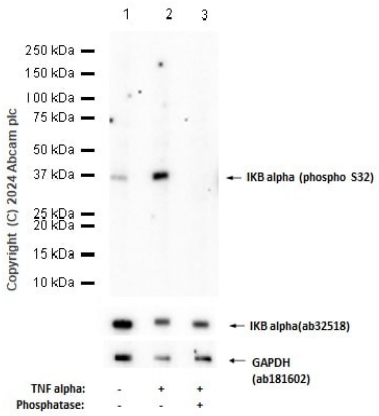
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

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

Exposure time: 20 seconds

Blocking/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 (**ab92700**).



Western blot - Anti-IKB alpha (phospho S32) antibody [EPR3148] - BSA and Azide free (ab239920)

All lanes : 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
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
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

Lysates/proteins at 20 µg per lane.

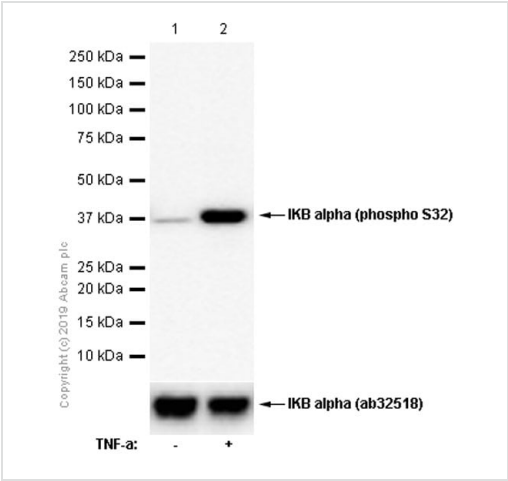
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

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

Exposure time: 180 seconds

Blocking/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 (**ab92700**).



Western blot - Anti-IKB alpha (phospho S32) antibody [EPR3148] - BSA and Azide free (ab239920)

All lanes : Anti-IKB alpha (phospho S32) antibody [EPR3148] (**ab92700**) at 1/1000 dilution (Purified)

Lane 1 : MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : MCF7 (Human breast adenocarcinoma epithelial cell) treated with 20 ng/mL TNF-alpha for 8 hours whole cell lysate

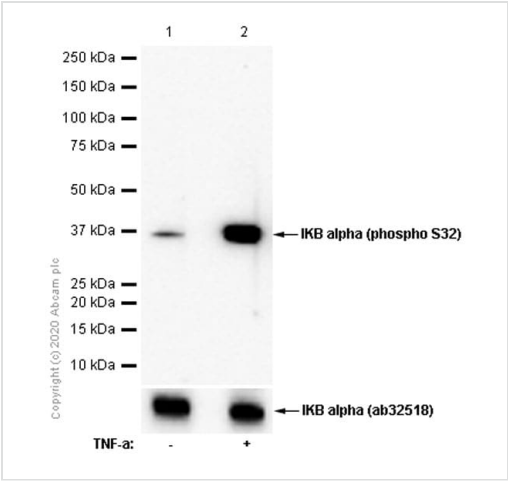
Lysates/proteins at 20 µg per lane.

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

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

Blocking/Diluting buffer: 5% NFDM/TBST

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab92700**).



Western blot - Anti-IKB alpha (phospho S32) antibody [EPR3148] - BSA and Azide free (ab239920)

All lanes : Anti-IKB alpha (phospho S32) antibody [EPR3148] (**ab92700**) at 1/1000 dilution (Purified)

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : HeLa (Human cervix adenocarcinoma epithelial cell) treated with 20 ng/mL TNF-alpha for 5 minutes whole cell lysate

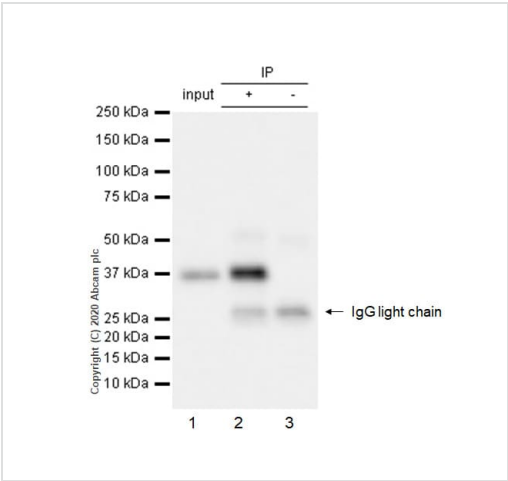
Lysates/proteins at 15 µg per lane.

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

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

Blocking/Diluting buffer: 5% NFDM/TBST

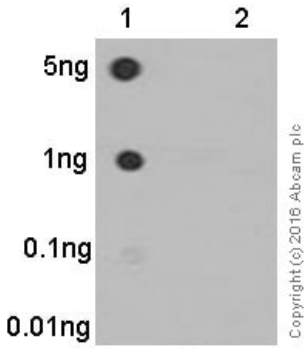
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab92700**).



Immunoprecipitation - Anti-IKB alpha (phospho S32) antibody [EPR3148] - BSA and Azide free (ab239920)

Purified **ab92700** at 1:20 dilution (1µg) immunoprecipitating IKB alpha in HeLa treated with 20ng/mL TNF-alpha for 60 minutes whole cell lysate.
Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) treated with 20ng/mL TNF-alpha for 60 minutes whole cell lysate 10µg
Lane 2 (+): **ab92700** + HeLa treated with 20ng/mL TNF-alpha for 60 minutes whole cell lysate.
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of **ab92700** in HeLa treated with 20ng/mL TNF-alpha for 60 minutes whole cell lysate.
Blocking Buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM/TBST.
Observed band size: 36 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab92700**).



Dot Blot - Anti-IKB alpha (phospho S32) antibody [EPR3148] - BSA and Azide free (ab239920)

Dot blot analysis of IKB alpha (phospho S32) phospho peptide (Lane 1) and IKB alpha non-phospho peptide (Lane 2) labeling IKB alpha (phospho S32) with unpurified **ab92700** at a dilution of 1/1000. **ab97051** (Peroxidase conjugated goat anti-rabbit IgG) (H+L) at 1/10000 was used as the secondary antibody.

Blocking and diluting buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab92700**).

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