abcam

Product datasheet

Anti-IKB alpha (phospho S32) antibody [EPR3148] ab92700

Recombinant RabMAb

41 References 8 Images

Overview

Product name	Anti-IKB alpha (phospho S32) antibody [EPR3148]
Description	Rabbit monoclonal [EPR3148] to IKB alpha (phospho S32)
Host species	Rabbit
Tested applications	**Suitable for:** Dot blot, WB, IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide within Human IKB alpha aa 1-100 (phospho S32). The exact sequence is proprietary. Database link: **P25963**
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	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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20

	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR3148
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab92700 in the following tested applications.

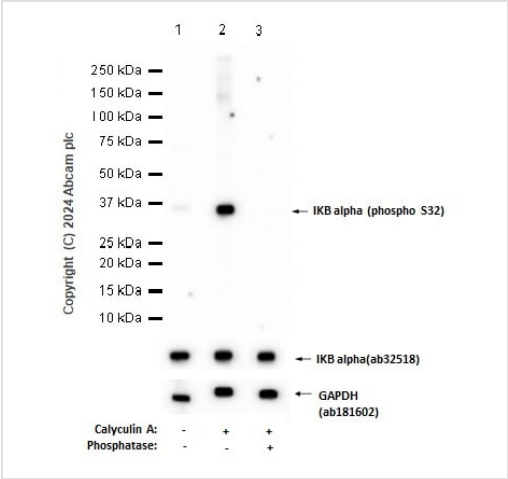
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Dot blot		1/1000.
WB		1/1000. Predicted molecular weight: 36 kDa. **For unpurified use at 1/500 - 1/10,000**
IP		1/20. **For unpurified use at 1/10 - 1/100**

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] (ab92700)

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

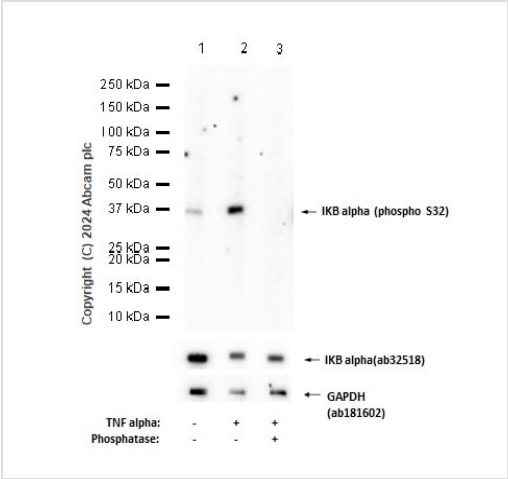
Lysates/proteins at 20 µg per lane.

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.



Western blot - Anti-IKB alpha (phospho S32) antibody [EPR3148] (ab92700)

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.

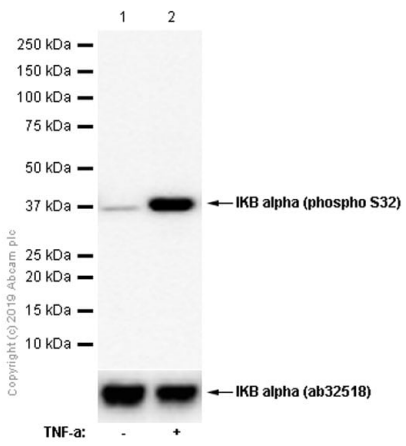
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000

dilution

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

Exposure time: 180 seconds

Blocking/Diluting buffer and concentration: 5% NFDM/TBST.



Western blot - Anti-IKB alpha (phospho S32) antibody [EPR3148] (ab92700)

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

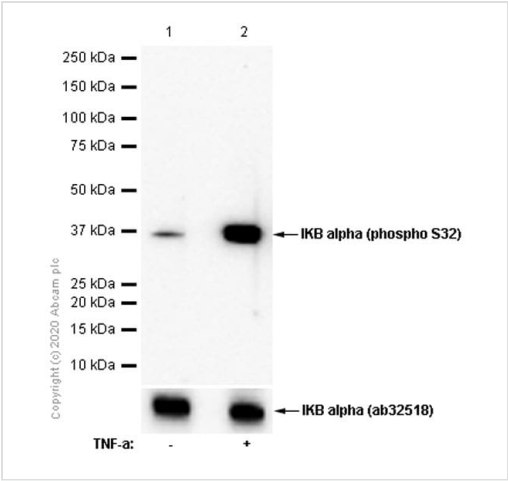
Lane 1 : MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : MCF7 (Human breast adenocarcinoma epithelial cell) treated with 20 ng/mL TNF-alpha for 8 hours whole cell lysate

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



Western blot - Anti-IKB alpha (phospho S32) antibody [EPR3148] (ab92700)

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

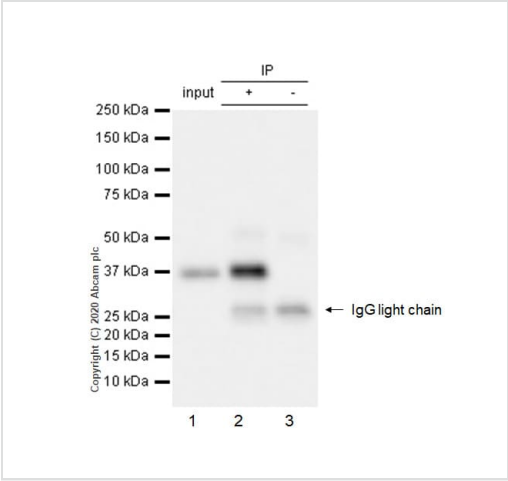
Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : HeLa (Human cervix adenocarcinoma epithelial cell) treated with 20 ng/mL TNF-alpha for 5 minutes whole cell lysate

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

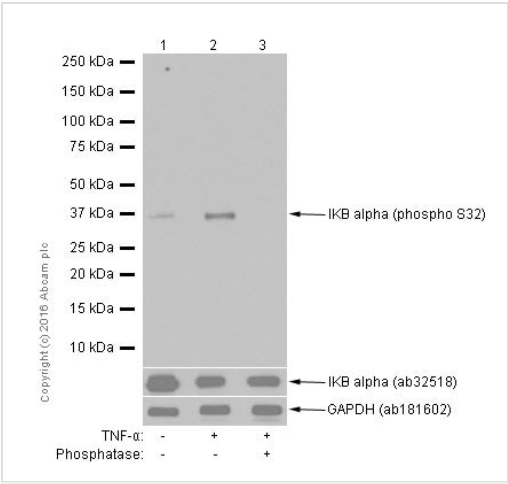


Immunoprecipitation - Anti-IKB alpha (phospho S32) antibody [EPR3148] (ab92700)

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



Western blot - Anti-IKB alpha (phospho S32) antibody [EPR3148] (ab92700)

All lanes : Anti-IKB alpha (phospho S32) antibody [EPR3148] (ab92700) at 1/500 dilution (unpurified)

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates
Lane 2 : HeLa (Human cervix adenocarcinoma epithelial cell) treated with TNF-a at 20 ng/mL for 5 minutes. Whole cell lysates
Lane 3 : HeLa treated with TNF-a at 20 ng/mL for 5 minutes. Whole cell lysates. Then the membrane was incubated with phosphatase.

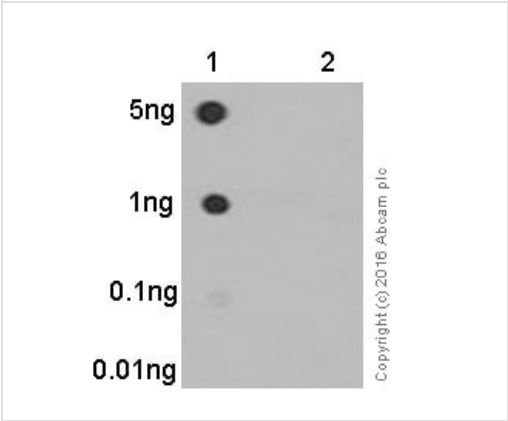
Lysates/proteins at 15 µg per lane.

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

Exposure time: 3 minutes

Blocking/Diluting buffer and concentration 5% NFDM/TBST



Dot Blot - Anti-IKB alpha (phospho S32) antibody [EPR3148] (ab92700)

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.

Anti-IKB alpha (phospho S32) antibody [EPR3148] (ab92700)

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