# abcam

# Product datasheet

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



重组 RabMAb

41 References 8 图像

概述

产品名称 Anti-IKB alpha (phospho S32)抗体[EPR3148]

描述 兔单克隆抗体[EPR3148] to IKB alpha (phospho S32)

宿主 Rabbit

经测试应用 适用于: Dot blot, WB, IP

种属反应性 与反应: Mouse, Rat, Human

免疫原 Synthetic peptide within Human IKB alpha aa 1-100 (phospho S32). The exact sequence is

proprietary.

Database link: P25963

阳性对照 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.

常规说明 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.

性能

形式

存放说明 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.

存储溶液 pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

纯**度** Protein A purified

**克隆** 单克隆

**克隆编号** EPR3148

**同种型** IgG

#### 应用

### The Abpromise guarantee Abpromise™承诺保证使用ab92700于以下的经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
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

10m	П	L-	-
蚆	1	7	K

功能 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.

疾病相关 Ectodermal dysplasia, anhidrotic, with T-cell immunodeficiency autosomal dominant

序列相似性 Belongs to the NF-kappa-B inhibitor family.

Contains 5 ANK repeats.

翻译后修饰 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.

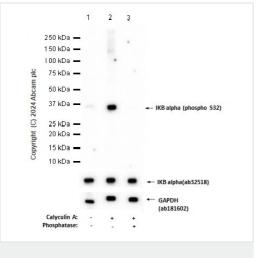
Deubiquitinated by porcine reproductive and respiratory syndrome virus Nsp2 protein, which

thereby interferes with NFKBIA degradation and impairs subsequent NF-kappa-B activation.

细胞定位 Cytoplasm. Nucleus. Shuttles between the nucleus and the cytoplasm by a nuclear localization

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

#### 图片



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) (<u>ab97051</u>) 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.

**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 virusinduced 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 virusinduced 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.

#### 3 250 kDa -150 kDa -100 kDa 🗕 75 kDa - . (C) 2024 Abcan 50 kDa -37 kDa 🕳 → IKB alpha (phospho S32) 25 kDa = 20 kDa = right 15 kDa -10 kDa - IKB alpha(ab32518) GAPDH (ab181602) TNF alpha

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

#### Secondary

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) 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.

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 lgG H&L (HRP) (ab97051) at 1/20000

dilution

Predicted band size: 36 kDa

Observed band size: 36 kDa

Blocking/Diluting buffer: 5% NFDM/TBST

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

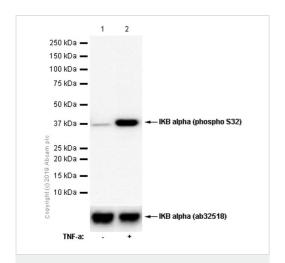
Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

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

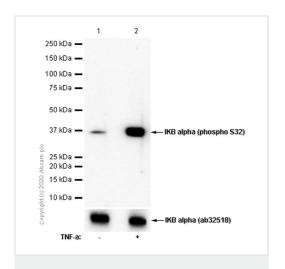
Lysates/proteins at 15 µg per lane.

Secondary

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



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



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

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

Blocking/Diluting buffer: 5% NFDM/TBST

IP

250 kDa —

150 kDa —

150 kDa —

100 kDa —

75 kDa —

50 kDa —

20 kDa —

20 kDa —

20 kDa —

20 kDa —

21 kDa —

22 kDa —

22 kDa —

23 kDa —

24 kDa —

25 kDa —

26 kDa —

26 kDa —

27 kDa —

28 kDa —

29 kDa —

40 kDa —

41 2 3

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

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

Purified ab92700 at 1:20 dilution (1 $\mu$ g) 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 (<u>ab172730</u>) 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

**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.

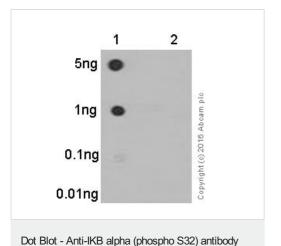
#### **Secondary**

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) 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



[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. <a href="mailto:ab97051">ab97051</a> (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.



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