# abcam

### Product datasheet

## Anti-IKB alpha antibody [EP697] ab76429



ילעבע RabMAb

★★★★★ 1 Abreviews 23 References 画像数8

#### 製品の概要

製品名 Anti-IKB alpha antibody [EP697]

製品の詳細 Rabbit monoclonal [EP697] to IKB alpha

由来種 Rabbit

アプリケーション 適用あり: WB, IHC-P, ICC/IF

適用なし: Flow Cyt

種交差性 交差種: Mouse, Rat, Human

免疫原 Synthetic peptide within Human IKB alpha (C terminal). The exact sequence is proprietary.

ポジティブ・コントロール HeLa, PC-12, RAW264.7 and Jurkat cell lysates; human kidney and colon tissue.

特記事項 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**.

#### 製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

バッファー pH: 7.20

Preservative: 0.01% Sodium azide

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

精製度 Protein A purified

ポリモノ モノクローナル

クローン名 EP697

ΙgG アイソタイプ

**The Abpromise guarantee** <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab76429の使用に適用されますアプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB	**** <u>(1)</u>	1/500 - 1/10000. Detects a band of approximately 36 kDa (predicted molecular weight: 36 kDa).
IHC-P		1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols. For unpurified use at 1/100 - 1/250.
ICC/IF		1/250. For unpurified use at 1/50 - 1/100.

追加情報

Is unsuitable for Flow Cyt.

#### ターゲット情報

機能

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.

関連疾患

配列類似性

翻訳後修飾

Ectodermal dysplasia, anhidrotic, with T-cell immunodeficiency autosomal dominant

Belongs to the NF-kappa-B inhibitor family.

Contains 5 ANK repeats.

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.

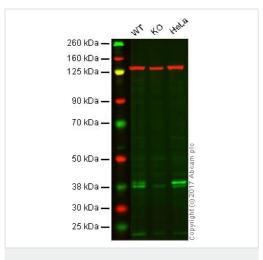
Deubiquitinated by porcine reproductive and respiratory syndrome virus Nsp2 protein, which thereby interferes with NFKBIA degradation and impairs subsequent NF-kappa-B activation.

細胞内局在

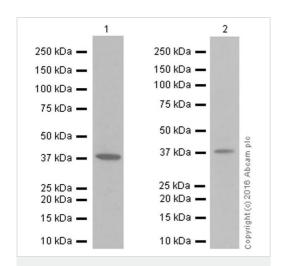
Cytoplasm. Nucleus. Shuttles between the nucleus and the cytoplasm by a nuclear localization

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

#### 画像



Western blot - Anti-IKB alpha antibody [EP697] (ab76429)



Western blot - Anti-IKB alpha antibody [EP697] (ab76429)

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 - ab76429 observed at 39 kDa. Red - loading control, **ab18058**, observed at 130 kDa.

ab76429 was shown to specifically react with IKB alpha in wild-type HAP1 cells as well as additional cross-reactive bands. No bands were observed when IKB alpha knockout cells were examined. Wild-type and IKB alpha knockout samples were subjected to SDS-PAGE. Ab76429 and <a href="mailto:ab18058">ab18058</a> (Mouse anti Vinculin loading control) were incubated overnight at 4°C at 1/500 dilution and 1/10,000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (<a href="mailto:ab216773">ab216773</a>) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (<a href="mailto:ab216776">ab216776</a>) secondary antibodies at 1/10,000 dilution for 1 hour at room temperature before imaging.

**All lanes :** Anti-IKB alpha antibody [EP697] (ab76429) at 1/1000 dilution

Lane 1 : Jurkat (human acute T cell leukemia) whole cell lysate

Lane 2 : HeLa (human cervix adenocarcinoma) whole cell lysate

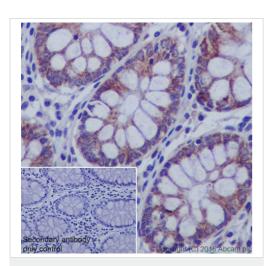
Lysates/proteins at 15 µg per lane.

#### **Secondary**

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

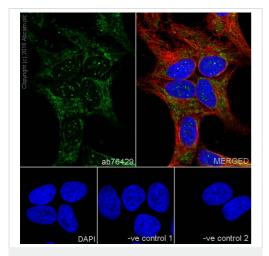
Predicted band size: 36 kDa Observed band size: 39 kDa

Blocking/Diluting buffer 5% NFDM /TBST



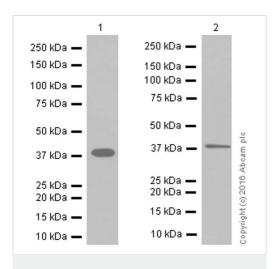
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-IKB alpha antibody
[EP697] (ab76429)

Immunohistochemical analysis of paraffin-embedded human colon tissue sections labelling IKB alpha with purified ab76429 at dilution of 1/500. Goat Anti-Rabbit IgG H&L (HRP) was used as the secondary antibody. The sample was counterstained with hematoxylin. Antigen retrieval was performed using 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 [EP697] (ab76429)

Immunocytochemistry/Immunofluorescence staining of HeLa cells labelling IKB alpha with purified ab76429 at a working dilution of 1/250. The secondary antibody was Alexa Fluor® 488 goat antirabbit (ab150077), used at a dilution of 1/1000. ab7291, a mouse anti-tubulin antibody (1/1000), was used to stain tubulin along with ab150120 (Alexa Fluor® 594 goat anti-mouse, 1/1000), shown in the top right hand panel. DAPI was used as nuclear counterstain. The cells were fixed in 4% Paraformaldehyde and permeabilized using 0.1% Triton X-100. The negative controls are shown in bottom middle and right hand panels - for negative control 1, rabbit primary antibody was used followed by an Alexa Fluor® 594 goat antimouse antibody (ab150120). For negative control 2, ab7291 (mouse anti-tubulin) was used followed by an Alexa Fluor® 488 goat anti-rabbit antibody (ab150077).



Western blot - Anti-IKB alpha antibody [EP697] (ab76429)

**All lanes :** Anti-IKB alpha antibody [EP697] (ab76429) at 1/10000 dilution

**Lane 1 :** RAW264.7 (mouse abelson murine leukemia virus-induced tumor) whole cell lysate

Lane 2 : PC-12 (rat adrenal gland pheochromocytoma) whole cell lysate

Lysates/proteins at 15 µg per lane.

#### **Secondary**

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/100000 dilution

**Predicted band size:** 36 kDa **Observed band size:** 39 kDa

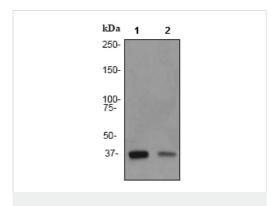
Blocking/Diluting buffer 5% NFDM /TBST



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-IKB alpha antibody
[EP697] (ab76429)

Immunohistochemical analysis of Paraffin-embedded human colon tissue section labelling IKB alpha with ab76429 at 1/100 dilution.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Western blot - Anti-IKB alpha antibody [EP697]

(ab76429)

**All lanes :** Anti-IKB alpha antibody [EP697] (ab76429) at 1/1000 dilution

Lane 1 : HeLa cell lysates

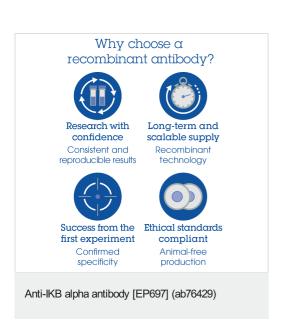
Lane 2 : Jurkat cell lysates

Lysates/proteins at 10 µg per lane.

#### Secondary

All lanes: goat anti-rabbit HRP at 1/1000 dilution

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



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