

## Product datasheet

# Anti-IKB alpha (phospho S36) antibody [EPR6235(2)] ab133462

Recombinant RabMAb

★★★★★ 1 Abreviews 196 References 4 Images

### Overview

<b>Product name</b>	Anti-IKB alpha (phospho S36) antibody [EPR6235(2)]
<b>Description</b>	Rabbit monoclonal [EPR6235(2)] to IKB alpha (phospho S36)
<b>Host species</b>	Rabbit
<b>Specificity</b>	This antibody detects IKB alpha only when phosphorylated at Serine 36. Stimulation may be required to allow detection of the phosphorylated protein due to low levels of endogenous expression. Please see images below for recommended treatment conditions and positive controls.
<b>Tested applications</b>	<b>Suitable for:</b> WB, Flow Cyt (Intra)
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Rat, Human
<b>Immunogen</b>	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
<b>Positive control</b>	HeLa cells treated with FBS and Calyculin A.
<b>General notes</b>	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> <li>- High batch-to-batch consistency and reproducibility</li> <li>- Improved sensitivity and specificity</li> <li>- Long-term security of supply</li> <li>- Animal-free production</li> </ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a>.</p>

### Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.
<b>Storage buffer</b>	<p>pH: 7.20</p> <p>Preservative: 0.01% Sodium azide</p> <p>Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA</p>
<b>Purity</b>	Protein A purified

<b>Clonality</b>	Monoclonal
<b>Clone number</b>	EPR6235(2)
<b>Isotype</b>	IgG

## Applications

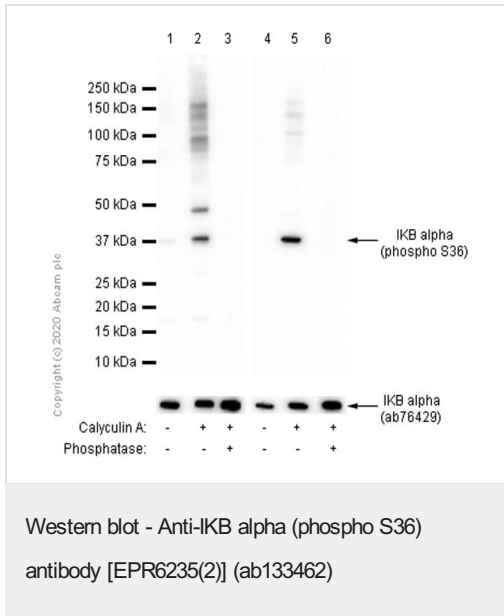
**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab133462 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
<b>WB</b>	★★★★★ (1)	1/10000. Detects a band of approximately 40 kDa (predicted molecular weight: 35 kDa).
<b>Flow Cyt (Intra)</b>		1/100.

## Target

<b>Function</b>	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.
<b>Involvement in disease</b>	Ectodermal dysplasia, anhidrotic, with T-cell immunodeficiency autosomal dominant
<b>Sequence similarities</b>	Belongs to the NF-kappa-B inhibitor family. Contains 5 ANK repeats.
<b>Post-translational modifications</b>	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.
<b>Cellular localization</b>	Cytoplasm. Nucleus. Shuttles between the nucleus and the cytoplasm by a nuclear localization signal (NLS) and a CRM1-dependent nuclear export.

## Images



**All lanes :** Anti-IκB alpha (phospho S36) antibody [EPR6235(2)] (ab133462) at 1/1000 dilution (Purified)

**Lane 1 :** NIH/3T3 (Mouse embryonic fibroblast) starved for 24 hours

**Lane 2 :** NIH/3T3 (Mouse embryonic fibroblast) starved for 24 hours then treated with 100nM Calyculin A for 30 minutes

**Lane 3 :** NIH/3T3 (Mouse embryonic fibroblast) starved for 24 hours then treated with 100nM Calyculin A for 30 minutes, then the membrane treated with Alkaline Phosphatase for 1 hour

**Lane 4 :** Untreated C6 (Rat glial tumor glial cell)

**Lane 5 :** C6 (Rat glial tumor glial cell) treated with 100ng/ml Calyculin A for 60 minutes

**Lane 6 :** C6 (Rat glial tumor glial cell) treated with 100ng/ml Calyculin A for 60 minutes, then the membrane treated with Alkaline Phosphatase for 1 hour

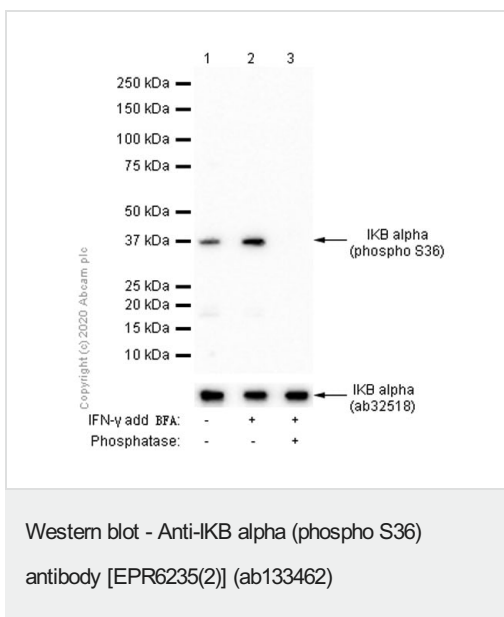
Lysates/proteins at 15 µg per lane.

## Secondary

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

**Predicted band size:** 35 kDa

**Observed band size:** 40 kDa



**All lanes :** Anti-IκB alpha (phospho S36) antibody [EPR6235(2)] (ab133462) at 1/1000 dilution (Purified)

**Lane 1 :** Untreated THP-1 (Human monocytic leukemia monocyte)

**Lane 2 :** THP-1 (Human monocytic leukemia monocyte) treated with 100ng/ml IFN-gamma for 24 hours, then 300ng/ml Brefeldin A added and treated for another 20 hours

**Lane 3 :** THP-1 (Human monocytic leukemia monocyte) treated with 100ng/ml IFN-gamma for 24 hours, then 300ng/ml Brefeldin A added and treated for another 20 hours, then the membrane treated with Alkaline Phosphatase for 1 hour

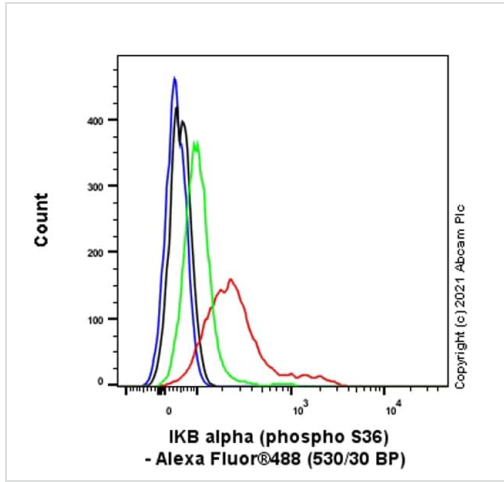
Lysates/proteins at 15 µg per lane.

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**Observed band size:** 40 kDa



Flow Cytometry (Intracellular) - Anti- $\text{IKB}$  alpha (phospho S36) antibody [EPR6235(2)] (ab133462)

Intracellular flow cytometric analysis of 4% Paraformaldehyde fixed 90% Methanol permeabilized HeLa (Human cervix adenocarcinoma epithelial cell) treated with 1% FBS for 3 hours then 100 nM Calyculin A for 10 minutes, labelling  $\text{IKB}$  alpha (phospho S36) with ab133462 at 1/100 dilution (1  $\mu\text{g/ml}$ ) compared with a Rabbit monoclonal IgG (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor<sup>®</sup> 488, **ab150077**) at 1/2000 was used as the secondary antibody. Untreated control: Green

#### Why choose a recombinant antibody?



Anti- $\text{IKB}$  alpha (phospho S36) antibody [EPR6235(2)] (ab133462)

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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