# abcam

# Product datasheet

# Anti-NF-kB p65 (phospho S536) antibody ab86299

\*\*\* 10 Abreviews 487 References 2 Images

Overview

Product name Anti-NF-kB p65 (phospho S536) antibody

**Description** Rabbit polyclonal to NF-kB p65 (phospho S536)

Host species Rabbit

**Tested applications** Suitable for: IHC-P, WB

Species reactivity Reacts with: Human

Predicted to work with: Guinea pig, Dog, Pig, Chimpanzee, Rhesus monkey, Gorilla,

Orangutan 4

**Immunogen** Synthetic peptide corresponding to Human NF-kB p65 (phospho S536).

Database link: Q04206

**General notes**The Life Science industry has been in the grips of a reproducibility crisis for a number of years.

Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

**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: 6.8

Preservative: 0.09% Sodium azide

Constituents: 0.1% BSA, Tris buffered saline

Purity Protein A purified

Purification notes ab86299 was affinity purified using an epitope specific to Phospho NFkB p65 (S536)

immobilized on solid support.

**Clonality** Polyclonal

**Isotype** IgG

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#### **Applications**

#### The Abpromise guarantee

Our Abpromise quarantee covers the use of ab86299 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
IHC-P	<b>★★★★</b> (1)	1/200 - 1/1000. Epitope retrieval with Tris-EDTA pH 9.0 is recommended for FFPE tissue sections.
WB	**** <u>(6)</u>	1/2000 - 1/10000. Predicted molecular weight: 60 kDa.

### **Target**

#### **Function**

NF-kappa-B is a pleiotropic transcription factor which is present in almost all cell types and is involved in many biological processed such as inflammation, immunity, differentiation, cell growth, tumorigenesis and apoptosis. NF-kappa-B is a homo- or heterodimeric complex formed by the Rel-like domain-containing proteins RELA/p65, RELB, NFKB1/p105, NFKB1/p50, REL and NFKB2/p52 and the heterodimeric p65-p50 complex appears to be most abundant one. The dimers bind at kappa-B sites in the DNA of their target genes and the individual dimers have distinct preferences for different kappa-B sites that they can bind with distinguishable affinity and specificity. Different dimer combinations act as transcriptional activators or repressors, respectively. NF-kappa-B is controlled by various mechanisms of post-translational modification and subcellular compartmentalization as well as by interactions with other cofactors or corepressors. NF-kappa-B complexes are held in the cytoplasm in an inactive state complexed with members of the NF-kappa-B inhibitor (I-kappa-B) family. In a conventional activation pathway, I-kappa-B is phosphorylated by I-kappa-B kinases (IKKs) in response to different activators, subsequently degraded thus liberating the active NF-kappa-B complex which translocates to the nucleus. NF-kappa-B heterodimeric p65-p50 and p65-c-Rel complexes are transcriptional activators. The NF-kappa-B p65-p65 complex appears to be involved in invasin-mediated activation of IL-8 expression. The inhibitory effect of I-kappa-B upon NF-kappa-B the cytoplasm is exerted primarily through the interaction with p65. p65 shows a weak DNA-binding site which could contribute directly to DNA binding in the NF-kappa-B complex. Associates with chromatin at the NF-kappa-B promoter region via association with DDX1.

### Sequence similarities

#### Domain

# Post-translational modifications

Contains 1 RHD (Rel-like) domain.

the 9aaTAD motif is a transactivation domain present in a large number of yeast and animal transcription factors.

Ubiquitinated, leading to its proteasomal degradation. Degradation is required for termination of NF-kappa-B response.

Monomethylated at Lys-310 by SETD6. Monomethylation at Lys-310 is recognized by the ANK repeats of EHMT1 and promotes the formation of repressed chromatin at target genes, leading to down-regulation of NF-kappa-B transcription factor activity. Phosphorylation at Ser-311 disrupts the interaction with EHMT1 without preventing monomethylation at Lys-310 and relieves the repression of target genes.

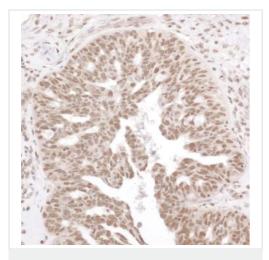
Phosphorylation at Ser-311 disrupts the interaction with EHMT1 and promotes transcription factor activity (By similarity). Phosphorylation on Ser-536 stimulates acetylation on Lys-310 and interaction with CBP; the phosphorylated and acetylated forms show enhanced transcriptional activity.

Reversibly acetylated; the acetylation seems to be mediated by CBP, the deacetylation by HDAC3. Acetylation at Lys-122 enhances DNA binding and impairs association with NFKBIA. Acetylation at Lys-310 is required for full transcriptional activity in the absence of effects on DNA binding and NFKBIA association. Acetylation can also lower DNA-binding and results in nuclear export. Interaction with BRMS1 promotes deacetylation of 'Lys-310'.

#### **Cellular localization**

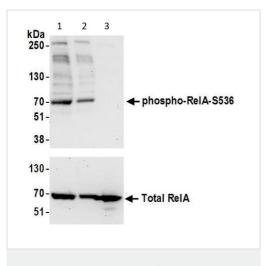
Nucleus. Cytoplasm. Nuclear, but also found in the cytoplasm in an inactive form complexed to an inhibitor (I-kappa-B). Colocalized with RELA in the nucleus upon TNF-alpha induction.

#### **Images**



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-NF-kB p65 (phospho S536) antibody (ab86299)

IHC image of NF-kB p65 (phospho S536) staining in Human ovarian carcinoma formalin fixed paraffin embedded tissue section, performed on a Leica BondTM system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab86299, 1µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.



Western blot - Anti-NF-kB p65 (phospho S536) antibody (ab86299)

**All lanes :** Anti-NF-kB p65 (phospho S536) antibody (ab86299) at 0.1 µg/ml

**Lane 1 :** Jurkat whole cell lysate treated with TNF alpha and Calyculin A at  $50~\mu g$ 

**Lane 2 :** Jurkat whole cell lysate treated with TNF alpha and Calyculin A at 15  $\mu g$ 

Lane 3: Jurkat whole cell lysate at 50 µg

Predicted band size: 60 kDa

**Detection:** Chemiluminescence with an exposure time of 30 seconds.

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

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