abcam

Product datasheet

Anti-NF-kB p65 (acetyl K310) antibody ab19870

★★★★★ 6 Abreviews 108 References 5 Images

Overview

Product name	Anti-NF-kB p65 (acetyl K310) antibody
Description	Rabbit polyclonal to NF-kB p65 (acetyl K310)
Host species	Rabbit
Tested applications	**Suitable for:** WB, IP, Dot blot **Unsuitable for:** ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat **Predicted to work with:** Human
Immunogen	Synthetic peptide corresponding to Human NF-kB p65 aa 300-400 (internal sequence) conjugated to keyhole limpet haemocyanin. (Peptide available as **ab20612**)
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. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.
Purity	Protein A purified

Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab19870 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
WB	★★★★☆ (3)	Use a concentration of 2.5 µg/ml. Detects a band of approximately 65 kDa (predicted molecular weight: 65 kDa). Collaborator data suggests that immunoprecipitation of this antibody prior to Western blotting is required to obtain the best results (see images)
IP		Use a concentration of 2.5 µg/ml.
Dot blot		Use at an assay dependent concentration.

Application notes Is unsuitable for ICC/IF.

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 Contains 1 RHD (Rel-like) domain.

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

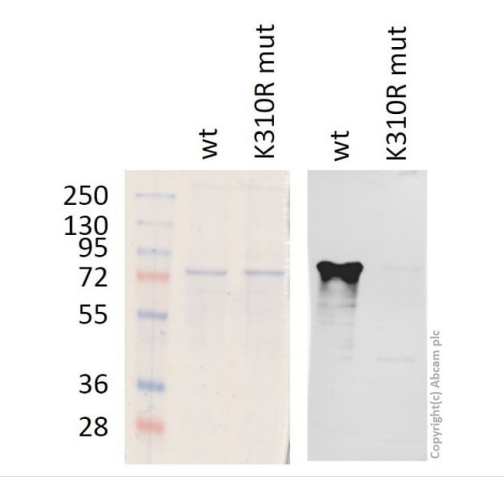
Post-translational modifications Ubiquitinated, leading to its proteasomal degradation. Degradation is required for termination of NF-kappa-B response.

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



Western blot - Anti-NF-kB p65 (acetyl K310) antibody (ab19870)

This image is courtesy of Prof. Michael Hottiger's Lab, University of Zurich

Lanes 1-2 : Coomassie stain

Lanes 3-4 : Anti-NF-kB p65 (acetyl K310) antibody (ab19870) at 1/500 dilution

Lanes 1 & 3 : Acetylated p65 protein

Lanes 2 & 4 : K310R mutant protein

Lysates/proteins at 2 µg per lane.

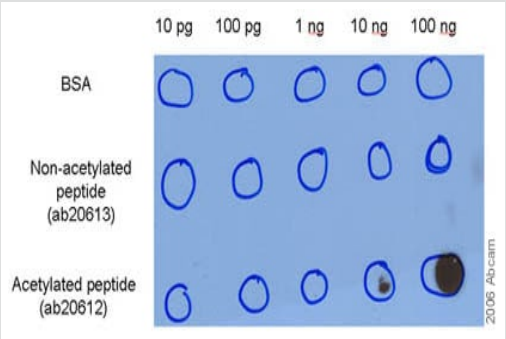
Secondary

Lanes 3-4 : IRDye® 800CW Goat anti-Rabbit IgG (H + L) at 1/15000 dilution

Predicted band size: 65 kDa

Observed band size: 75kDa

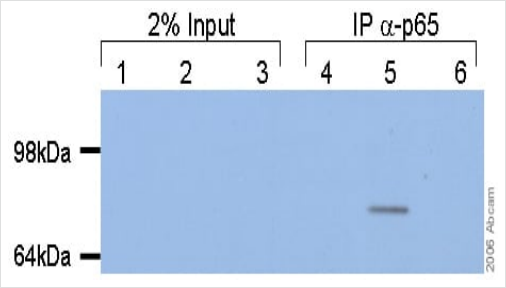
The p65 band runs higher in this blot because the protein contains a myc-tag.



Dot Blot - Anti-NF-kB p65 (acetyl K310) antibody (ab19870)

This image is courtesy of Christine Buerki and Karin Rothgiesser, University of Zurich, Switzerland

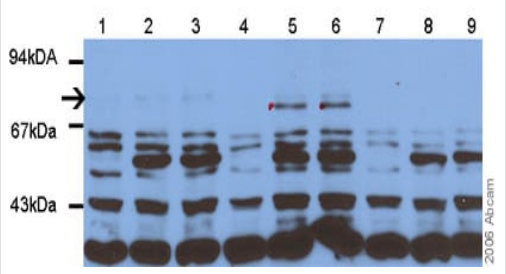
Rabbit polyclonal to NF-kB p65 (acetyl K310) (ab19870; 2.5µg/ml) in 1% non-fat milk TBS-T incubated for 3h at room temperature. Exposure time: 75 min normal ECL. This Dot blot demonstrates that ab19870 recognized upto 10ng of purified peptide on a PVDF membrane.



Immunoprecipitation - Anti-NF-kB p65 (acetyl K310) antibody (ab19870)

This image is courtesy of Christine Buerki and Karin Rothgiesser, University of Zurich, Switzerland

Western Blot with ab19870 after p65 Immunoprecipitation: rabbit polyclonal to NF-kB p65 (acetyl K310) (ab19870; 2.5µg/ml) in 1% non-fat milk TBS-T incubated for 3 hours at room temperature. Exposure time: 1 min normal ECL. Tested samples: nuclear extracts (180 µg) of immortalized p65-/- mouse cells, complemented with the empty vector (pRRL), wild-type p65 (Wt) and non-acetylatable K310 (K310R). The samples tested were treated with deacetylase inhibitors HDACi (TSA + Nicotinamide) and TNF-alpha. The samples were immunoprecipitated with 2µg of alpha-p65 and subsequently analysed by Western blot with Rabbit polyclonal to NF-kB p65 (acetyl K310) (ab19870). Predicted band size = 65kDa, Observed band size = 75kDa. The p65 band runs higher in this SDS-PAGE blot as it contains a myc-tag.



Western blot - Anti-NF-kB p65 (acetyl K310) antibody (ab19870)

This image is courtesy of Christine Buerki and Karin Rothgiesser, University of Zurich, Switzerland

All lanes : Anti-NF-kB p65 (acetyl K310) antibody (ab19870) at 2.5 µg/ml

Lane 1 : pRRL untreated
Lane 2 : pRRL HDACi
Lane 3 : pRRL HDACi + TNF
Lane 4 : Wt untreated
Lane 5 : Wt HDACi
Lane 6 : Wt HDACi + phorbol myristate acetate
Lane 7 : K310R untreated
Lane 8 : K310R HDACi
Lane 9 : K310R HDACi + phorbol myristate acetate

Lysates/proteins at 75 µg per lane.

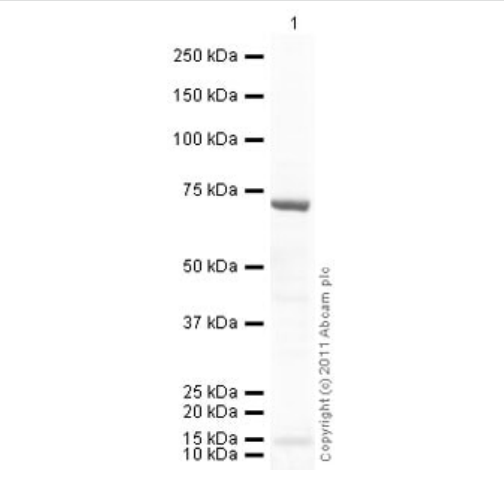
Developed using the ECL technique.

Predicted band size: 65 kDa
Observed band size: 75 kDa

Exposure time: 1 hour

ab19870 recognizes Rabbit polyclonal to NF-kB p65 (acetyl K310) specifically at ~75kDa (indicated by the arrow) is this SDS-PAGE blot. The p65 band runs higher than 65kDa in this SDS-PAGE blot as it contains a myc-tag. We are sure that the band at ~75kDa is p65 since p65 specific antibodies detect the same band in IP and WB and there is no signal in the p65 knock-out cell line with ab19870. A number of additonal bands are recognized by ab19870 when tested with endogenous p65 from whole cell extracts, we do not know the identity of these bands.

Tested samples: nuclear extracts (75µg) of immortalized p65-/- mouse cells, complemented with the empty ve



Western blot - Anti-NF-kB p65 (acetyl K310) antibody (ab19870)

Anti-NF-kB p65 (acetyl K310) antibody (ab19870) at 1 µg/ml + Lung (Rat) Tissue Lysate at 10 µg

Secondary
Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (**ab97080**) at 1/5000 dilution

Performed under reducing conditions.

Predicted band size: 65 kDa
Observed band size: 72 kDa
Additional bands at: 15 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 4 minutes

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