

## Product datasheet

# Anti-NF-kB p65 (acetyl K310) antibody - ChIP Grade ab19870

★★★★★ 6 Abreviews 47 References 6 Images

### Overview

<b>Product name</b>	Anti-NF-kB p65 (acetyl K310) antibody - ChIP Grade
<b>Description</b>	Rabbit polyclonal to NF-kB p65 (acetyl K310) - ChIP Grade
<b>Host species</b>	Rabbit
<b>Tested applications</b>	<b>Suitable for:</b> WB, IP, Dot blot, ICC, ChIP
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Rat, Human
<b>Immunogen</b>	Synthetic peptide corresponding to Human NF-kB p65 aa 300-400 (internal sequence) conjugated to Keyhole Limpet Haemocyanin (KLH). (Peptide available as <a href="#">ab20612</a> )
<b>Positive control</b>	This antibody gave a positive signal in Rat lung tissue lysate.

### Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
<b>Storage buffer</b>	Preservative: 0.02% Sodium Azide Constituents: 1% BSA, PBS, pH 7.4
<b>Purity</b>	Protein A purified
<b>Clonality</b>	Polyclonal
<b>Isotype</b>	IgG

### Applications

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
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Application	Abreviews	Notes
WB	★★★★☆	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 dilution.
ICC		Use at an assay dependent dilution. In ICC/IF ab19870 recognizes various acetylated nuclear protein(s), as the signal is also observed in control cells; the signal in ICC is HDACi-dependent.
ChIP		Use at an assay dependent concentration. PubMed: 22249179

## Target

### Function

NF-kappa-B is a pleiotropic transcription factor which is present in almost all cell types and is involved in many biological processes 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 in 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

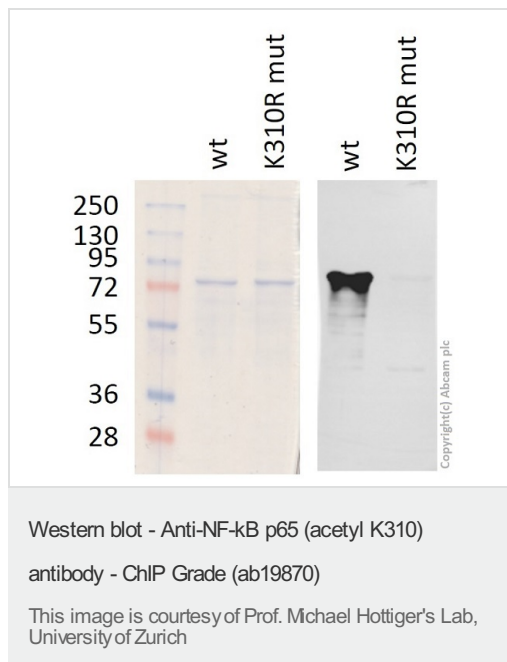
interaction with CBP; the phosphorylated and acetylated forms show enhanced transcriptional activity.

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



**Lanes 1-2 :** Coomassie stain

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

**Lanes 1 & 3 :** Acetylated p65 protein

**Lanes 2 & 4 :** K310 mutant protein

Lysates/proteins at 2 µg per lane.

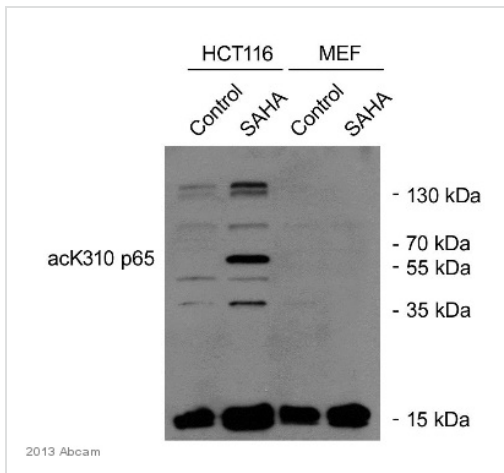
### Secondary

**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.



Western blot - Anti-NF-κB p65 (acetyl K310) antibody - ChIP Grade (ab19870)

This image is courtesy of an Abreview submitted by Christian Marx

**All lanes :** Anti-NF-κB p65 (acetyl K310) antibody - ChIP Grade (ab19870) at 1/1000 dilution

**Lane 1 :** HCT116 whole cell lysate treated with DMSO for 24 hrs (control)

**Lane 2 :** HCT116 whole cell lysate treated with 2 μM SAHA for 24 hrs

**Lane 3 :** MEF whole cell lysate treated with DMSO for 24 hrs (control)

**Lane 4 :** MEF whole cell lysate treated with 2 μM SAHA for 24 hrs

Lysates/proteins at 60 μg per lane.

### Secondary

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/10000 dilution

Developed using the ECL technique.

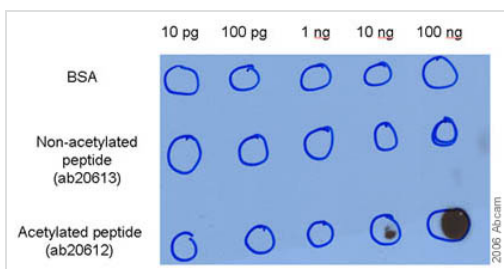
Performed under reducing conditions.

**Predicted band size:** 65 kDa

**Observed band size:** 65 kDa

**Additional bands at:** 140 kDa (possible non-specific binding), 15 kDa (possible non-specific binding), 40 kDa (possible non-specific binding), 45 kDa (possible non-specific binding), 90 kDa (possible non-specific binding)

**Exposure time:** 2 minutes

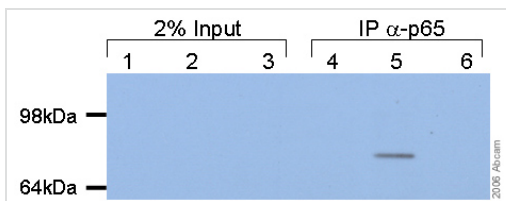


Dot Blot - Anti-NF-κB p65 (acetyl K310) antibody - ChIP Grade (ab19870)

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

Rabbit polyclonal to NF-κB p65 (acetyl K310) (ab19870; 2.5 μg/ml) in 1% non-fat milk TBS-T incubated for 3h at room temperature.

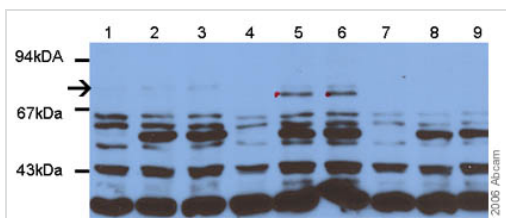
Exposure time: 75 min normal ECL. This Dot blot demonstrates that ab19870 recognized upto 10ng of purified peptide on a PVDF membrane.



Immunoprecipitation - Anti-NF-kB p65 (acetyl K310) antibody - ChIP Grade (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<sup>-/-</sup> mouse cells, complemented with the empty vector (pRRL), wild-type p65 (Wt) and non-acetyltable 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 - ChIP Grade (ab19870)

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

**All lanes :** Anti-NF-kB p65 (acetyl K310) antibody - ChIP Grade (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

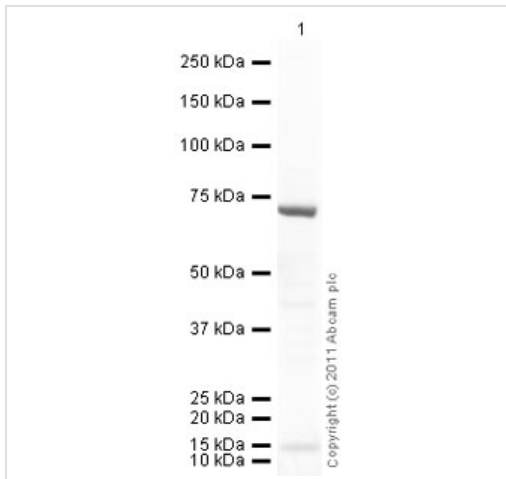
[why is the actual band size different from the predicted?](#)

**Exposure time:** 1 hour

ab19870 recognizes Rabbit polyclonal to NF-kB p65 (acetyl K310) specifically at ~75kDa (indicated by the arrow) in 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 additional 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<sup>-/-</sup> mouse cells, complemented with the empty ve



Western blot - Anti-NF-κB p65 (acetyl K310) antibody - ChIP Grade (ab19870)

Anti-NF-κB p65 (acetyl K310) antibody - ChIP Grade (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 [why is the actual band size different from the predicted?](#)

**Additional bands at:** 15 kDa. We are unsure as to the identity of these extra bands.

**Exposure time:** 4 minutes

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

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