# abcam

# Product datasheet

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

★★★★★ 6 Abreviews 108 References 画像数 5

#### 製品の概要

製品名 Anti-NF-kB p65 (acetyl K310) antibody

製品の詳細 Rabbit polyclonal to NF-kB p65 (acetyl K310)

由来種 Rabbit

アプリケーション 適用あり: WB, IP, Dot blot

適用なし: ICC/IF

種交差性 交差種: Mouse, Rat

交差が予測される動物種: Human 🗥

免疫原 Synthetic peptide corresponding to Human NF-kB p65 aa 300-400 (internal sequence)

conjugated to keyhole limpet haemocyanin.

(Peptide available as ab20612)

特記事項 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

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: 7.40

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.

精製度 Protein A purified

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ポリモノ

ポリクローナル

アイソタイプ

ΙgG

#### アプリケーション

**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ab19870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	<b>★★★★☆</b> (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.

追加情報

Is unsuitable for ICC/IF.

#### ターゲット情報

#### 機能

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.

配列類似性

Contains 1 RHD (Rel-like) domain.

ドメイン

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

翻訳後修飾

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.

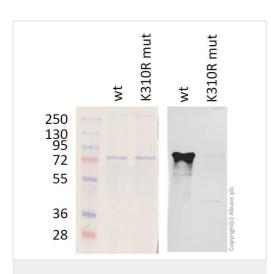
Phosphorylation at Ser-311 disrupts the interaction with EHMT1 and promotes transcription factor activity (By similarity). Phosphorylation on Ser-536 stimulates acetylation on Lys-310 and interaction with CBP; the phosphorylated and acetylated forms show enhanced transcriptional activity.

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'.

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.

# 細胞内局在

#### 画像



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

1/300 dilation

Lanes 1 & 3 : Acetylated p65 protein
Lanes 2 & 4 : K310R mutant protein

Lysates/proteins at 2 µg per lane.

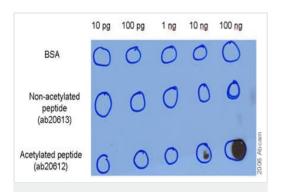
Secondary

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.

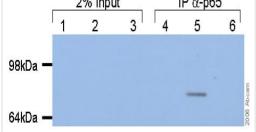


Rabbit polyclonal to NF-kB p65 (acetyl K310) (ab19870; 2.5µg/ml) in 1% non-fat milk TBS-T incubated for 3h at room temperature. Exposure time: 75 min normal ECL. This Dot blot demonstrates that ab19870 recognized upto 10ng of purified peptide on a PVDF membrane.



Rothgiesser, University of Zurich, Switzerland

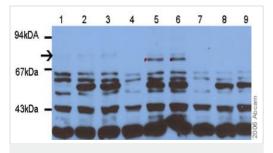




Immunoprecipitation - Anti-NF-kB p65 (acetyl K310) antibody (ab19870)

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;  $2.5\mu g/ml$ ) 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  $\mu g$ ) 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 2 $\mu g$  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  $\mu g/ml$ 

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 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-/mouse cells, complemented with the empty ve

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

#### Secondary

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

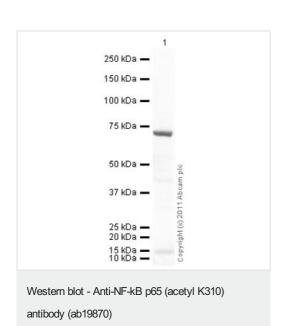
Performed under reducing conditions.

**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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