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

Overview

Product name
Anti-NF-kB p65 (acetyl K310) antibody - ChIP Grade

Description
Rabbit polyclonal to NF-kB p65 (acetyl K310) - ChIP Grade

Host species
Rabbit

Tested applications
Suitable for: WB, IP, Dot blot, ICC, ChIP

Species reactivity
Reacts with: Mouse, Rat, Human

Immunogen
Synthetic peptide corresponding to Human NF-kB p65 aa 300-400 (internal sequence) conjugated to Keyhole Limpet Haemocyanin (KLH). (Peptide available as ab20612)

Positive control
This antibody gave a positive signal in Rat lung tissue lysate.

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
Preservative: 0.02% Sodium Azide
Constituents: 1% BSA, PBS, pH 7.4

Purity
Protein A purified

Clonality
Polyclonal

Isotype
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.

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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

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<td>WB</td>
<td>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)</td>
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<tr>
<td>IP</td>
<td>Use a concentration of 2.5 µg/ml.</td>
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<tr>
<td>Dot blot</td>
<td>Use at an assay dependent dilution.</td>
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<tr>
<td>ICC</td>
<td>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.</td>
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<tr>
<td>ChIP</td>
<td>Use at an assay dependent concentration. PubMed: 22249179</td>
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Target

Application Abreviews Notes

Abreviews

Notes

Use at an assay dependent dilution.

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 - ChIP Grade (ab19870)**

This image is courtesy of an Abreview submitted by Christian Marx

<table>
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<th>Lysates/proteins at 60 µg per lane.</th>
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<tr>
<td>Lanes:</td>
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<tr>
<td>All lanes: Anti-NF-kB p65 (acetyl K310) antibody - ChIP Grade (ab19870) at 1/1000 dilution</td>
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<tr>
<td>Lane 1: HCT116 whole cell lysate treated with DMSO for 24 hrs (control)</td>
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<tr>
<td>Lane 2: HCT116 whole cell lysate treated with 2 µM SAHA for 24 hrs</td>
</tr>
<tr>
<td>Lane 3: MEF whole cell lysate treated with DMSO for 24 hrs (control)</td>
</tr>
<tr>
<td>Lane 4: MEF whole cell lysate treated with 2 µM SAHA for 24 hrs</td>
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**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
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 up to 10ng of purified peptide on a PVDF membrane.

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.
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 NFkB 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 vector.
Anti-NF-kB 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  
**Additional bands at:** 15 kDa. We are unsure as to the identity of these extra bands.

**Exposure time:** 4 minutes

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