abcam

Product datasheet

Anti-NF-kB p65 (acetyl K310) antibody [EPR21781] - BSA and Azide free ab237591

Recombinant RabMAb

2 References 4 Images

Overview

Product name	Anti-NF-kB p65 (acetyl K310) antibody [EPR21781] - BSA and Azide free
Description	Rabbit monoclonal [EPR21781] to NF-kB p65 (acetyl K310) - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Dot blot, ChIP, WB
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide within Human NF-kB p65 aa 300-400 (acetyl K310). The exact sequence is proprietary. Database link: Q04206
Positive control	WB: HEK-293 transfected with NF-kB p65 and p300 (aa1287-1663) expression vectors containing a myc-His-tag®, then treated with 20 ng/ml TNF-alpha for 60 minutes whole cell lysate.
General notes	Ab237591 is the carrier-free version of ab218533. This format is designed for use in antibody labeling, including fluorochromes, metal isotopes, oligonucleotides, enzymes. Our carrier-free formats are supplied in a buffer free of BSA, sodium azide and glycerol for higher conjugation efficiency. Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. ab237591 is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm. *Maxpar® is a trademark of Fluidigm Canada Inc.* This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information see here. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

Reproducibility is key to advancing scientific discovery and accelerating scientists' next breakthrough.

Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility.

We are also planning to innovate the way in which we present recommended applications and species on our product datasheets, so that only applications & species that have been tested in our own labs, our suppliers or by selected trusted collaborators are covered by our Abpromise™ guarantee.

In preparation for this, we have started to update the applications & species that this product is Abpromise guaranteed for.

We are also updating the applications & species that this product has been "predicted to work with," however this information is not covered by our Abpromise guarantee.

Applications & species from publications and Abreviews that have not been tested in our own labs or in those of our suppliers are not covered by the Abpromise guarantee.

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, as well as customer reviews and Q&As.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR21781
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab237591** in the following tested applications.

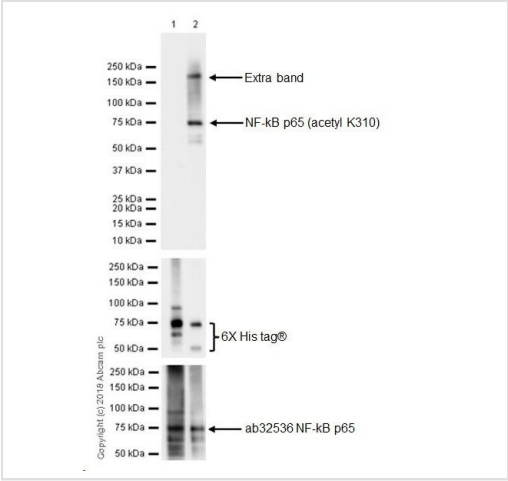
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Dot blot		Use at an assay dependent concentration.
ChIP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 70 kDa (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	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 antibody [EPR21781] - BSA and Azide free (ab237591)

All lanes : Anti-NF-kB p65 (acetyl K310) antibody [EPR21781] - ChIP Grade (ab218533) at 1/2000 dilution

Lane 1 : HEK-293 transfected with NF-kB p65 expression vector containing a myc-His-tag®, whole cell lysate
Lane 2 : HEK-293 transfected with NF-kB p65 and p300 (aa1287-1663) expression vectors containing a myc-His-tag®, then treated with 20 ng/ml TNF-alpha for 60 minutes, whole cell lysate

Lysates/proteins at 10 µg per lane.

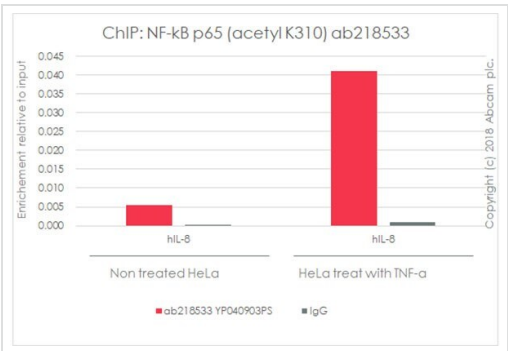
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 60 kDa
Observed band size: 70 kDa
why is the actual band size different from the predicted?

Blocking/Dilution buffer: 5% NFDM/TBST.

NF-κB p65 (acetyl K310) expression is induced by TNF-α and p300 acetyltransferases (PMID: 20160011, PMID: 12456660, PMID: 16135789).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab218533).

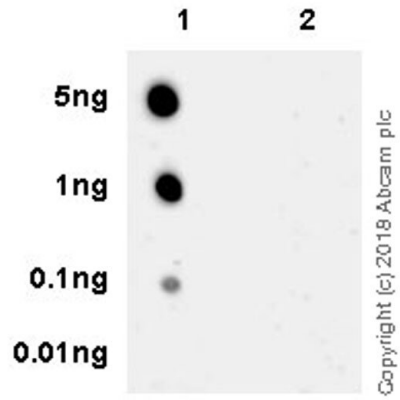


ChIP - Anti-NF-kB p65 antibody [EPR21781] - BSA and Azide free (ab237591)

Chromatin was prepared from HeLa (human epithelial cell line from cervix adenocarcinoma) cells treated with and without 20 ng/ml TNF-α for 60 minutes according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25 µg of chromatin, 5 µg of ab218533 (red), and 20 µl of Protein A/G sepharose beads. 5 µg of rabbit normal IgG was added to the beads control (gray). The immunoprecipitated DNA was quantified by real time PCR (SYBR green approach).

The ChIP data are consistent with the literature (PMID: 16135789).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab218533).



Dot Blot - Anti-NF-kB p65 antibody [EPR21781] - BSA and Azide free (ab237591)

Dot blot analysis of NF-kB p65 (acetyl K310) labeled with ab218533 at 1/1000 dilution.

Lane 1: NF-κB p65 (acetyl K310) peptide.

Lane 2: NF-κB p65 non-acetyl peptide.

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution was used as secondary antibody.

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 2 minutes.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab218533).

Anti-NF-kB p65 (acetyl K310) antibody [EPR21781] - BSA and Azide free (ab237591)

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