abcam

Product datasheet

Anti-NF-kB p65 antibody [EP2161Y] ab76311

KO VALIDATED Recombinant RabMAb

4 References 3 Images

Overview

Product name	Anti-NF-kB p65 antibody [EP2161Y]
Description	Rabbit monoclonal [EP2161Y] to NF-kB p65
Specificity	ab76311 detects both phosphorylated and non-phosphorylated versions of human NF-kB p65.
Tested applications	**Suitable for:** WB, Flow Cyt, ICC/IF **Unsuitable for:** IHC-P or IP
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide (the amino acid sequence is considered to be commercially sensitive) corresponding to Human NF-kB p65.
Positive control	HeLa, Daudi and Jurkat cell lysates.
General notes	A trial size is available to purchase for this antibody. Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents This product is a recombinant rabbit monoclonal antibody.

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	PBS 49%,Sodium azide 0.01%,Glycerol 50%,BSA 0.05%
Purity	Tissue culture supernatant
Clonality	Monoclonal
Clone number	EP2161Y
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab76311** 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		1/1000 - 1/5000. Detects a band of approximately 70 kDa (predicted molecular weight: 65 kDa).
Flow Cyt		1/30. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/100 - 1/500.

Application notes Is unsuitable for IHC-P or IP.

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.

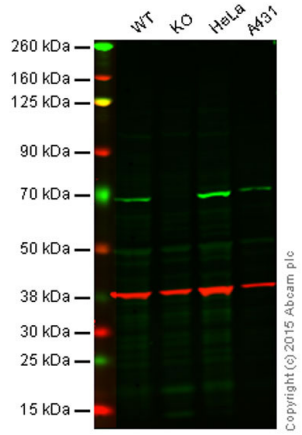
Post-translational modifications Ubiquitinated, leading to its proteasomal degradation. Degradation is required for termination of NF-kappa-B response.
Monomethylated at Lys-310 by SETD6. Monomethylation at Lys-310 is recognized by the ANK repeats of EHMT1 and promotes the formation of repressed chromatin 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

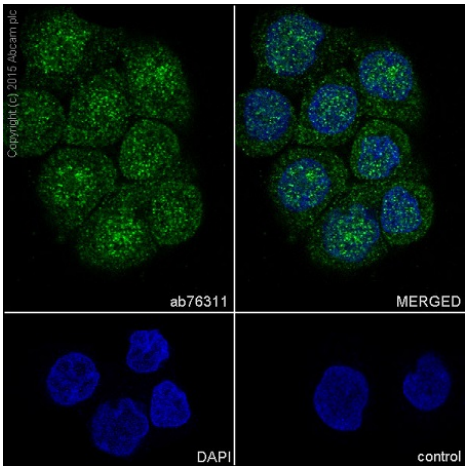


Western blot - Anti-NF-kB p65 antibody [EP2161Y] (ab76311)

Predicted band size : 65 kDa

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: NF-κB p65 knockout HAP1 cell lysate (20 µg)
Lane 3: HeLa cell lysate (20 µg)
Lane 4: A431 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab76311 observed at 70 kDa. Red - ab8245 loading control, observed at 37 kDa.

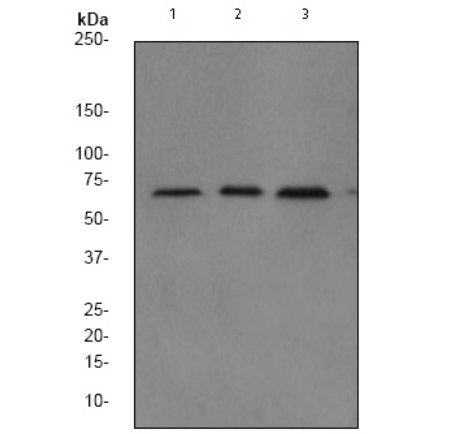
ab76311 was shown to specifically react with NF-κB p65 in wild-type HAP1 cells along with additional cross-reactive bands. No band was observed when NF-κB p65 knockout samples were used. Wild-type and NF-κB p65 knockout samples were subjected to SDS-PAGE. ab76311 (NF-κB p65) and ab8245 (loading control to GAPDH) were both diluted 1/1000 and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1/10000 dilution for 1 h at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-NF-kB p65 antibody [EP2161Y] (ab76311)

Immunocytochemistry/Immunofluorescence analysis HT-29 (human colorectal adenocarcinoma) labelling NF-kB p65 with purified ab76311 at 1/500. Cells were fixed with 4% PFA and permeabilized with 0.1% Triton X-100. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody (Ab150077). Nuclei counterstained with DAPI (blue).

Control: PBS only



Western blot - Anti-NF-kB p65 [EP2161Y] antibody (ab76311)

All lanes : Anti-NF-kB p65 antibody [EP2161Y] (ab76311) at 1/20000 dilution

Lane 1 : HeLa cell lysate
Lane 2 : Daudi cell lysate
Lane 3 : Jurkat cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size : 65 kDa
Observed band size : 70 kDa

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