

Product datasheet

Anti-NF-kB p65 antibody [EP2161Y] ab76311

KO VALIDATED Recombinant RabMAB[®]

[6 References](#) [3 Images](#)

Overview

Product name	Anti-NF-kB p65 antibody [EP2161Y]
Description	Rabbit monoclonal [EP2161Y] to NF-kB p65
Host species	Rabbit
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 within Human NF-kB p65. The exact sequence is proprietary.
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](#)

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

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	Protein A purified
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 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 invasion-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.

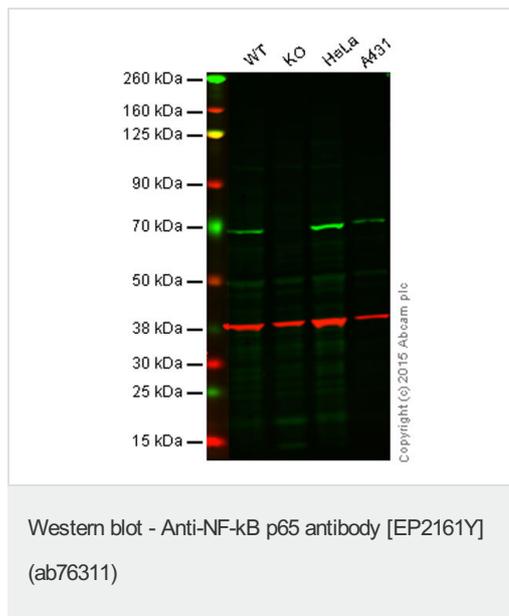
Phosphorylation at Ser-311 disrupts the interaction with EHMT1 and promotes transcription factor activity (By similarity). Phosphorylation on Ser-536 stimulates acetylation on Lys-310 and interaction with CBP; the phosphorylated and acetylated forms show enhanced transcriptional activity.

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



Lane 1: Wild-type HAP1 cell lysate (20 µg)

Lane 2: NF-κB p65 knockout HAP1 cell lysate (20 µg)

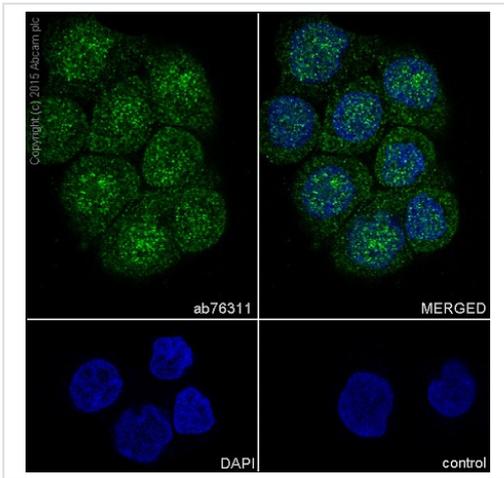
Lane 3: HeLa cell lysate (20 µg)

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.

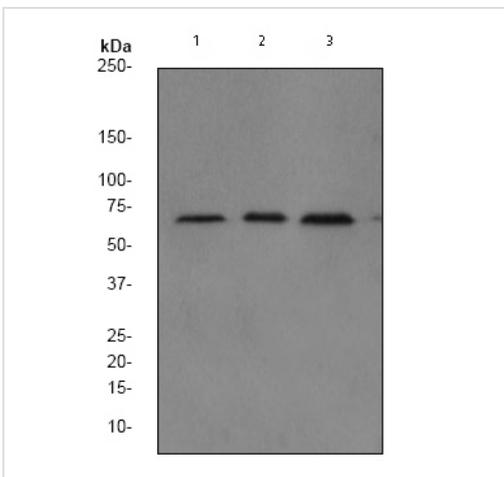
ab76311 was shown to specifically react with NF-κB p65 in wild-type HAP1 cells along with additional cross-reactive bands. No band was observed when NF-κB p65 knockout samples were used. Wild-type and NF-κB p65 knockout samples were subjected to SDS-PAGE. ab76311 (NF-κB p65) and ab8245 (loading control 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 antibody [EP2161Y] (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

All lanes : HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 65 kDa

Observed band size: 70 kDa

Please note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

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