

## Product datasheet

# Anti-NF-kB p65 antibody [EP2161Y] ab76311

**KO VALIDATED** Recombinant RabMAB<sup>®</sup>

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

### Overview

<b>Product name</b>	Anti-NF-kB p65 antibody [EP2161Y]
<b>Description</b>	Rabbit monoclonal [EP2161Y] to NF-kB p65
<b>Specificity</b>	ab76311 detects both phosphorylated and non-phosphorylated versions of human NF-kB p65.
<b>Tested applications</b>	<b>Suitable for:</b> WB, Flow Cyt, ICC/IF <b>Unsuitable for:</b> IHC-P or IP
<b>Species reactivity</b>	<b>Reacts with:</b> Human
<b>Immunogen</b>	Synthetic peptide (the amino acid sequence is considered to be commercially sensitive) corresponding to Human NF-kB p65.
<b>Positive control</b>	HeLa, Daudi and Jurkat cell lysates.
<b>General notes</b>	

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<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to [RabMab<sup>®</sup> patents](#)

This product is a recombinant rabbit monoclonal antibody.

### Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
<b>Storage buffer</b>	PBS 49%,Sodium azide 0.01%,Glycerol 50%,BSA 0.05%
<b>Purity</b>	Tissue culture supernatant
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	EP2161Y
<b>Isotype</b>	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. <a href="#">ab172730</a> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/100 - 1/500.
<b>Application notes</b>		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.

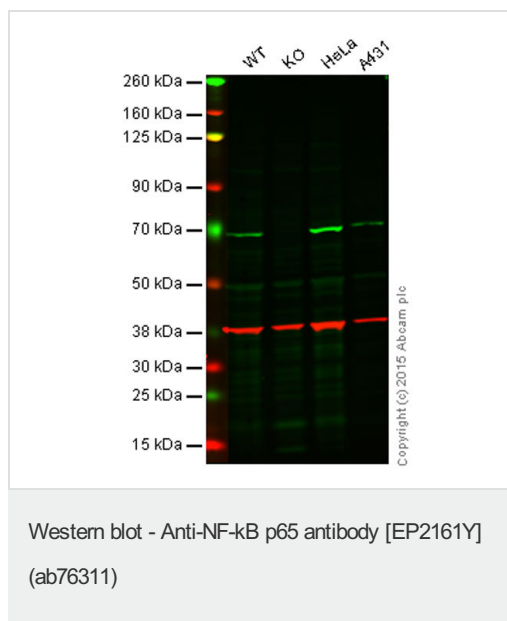
Phosphorylation at Ser-311 disrupts the interaction with EHMT1 and promotes transcription factor activity (By similarity). Phosphorylation on Ser-536 stimulates acetylation on Lys-310 and interaction with CBP; the phosphorylated and acetylated forms show enhanced transcriptional activity.

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



**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)

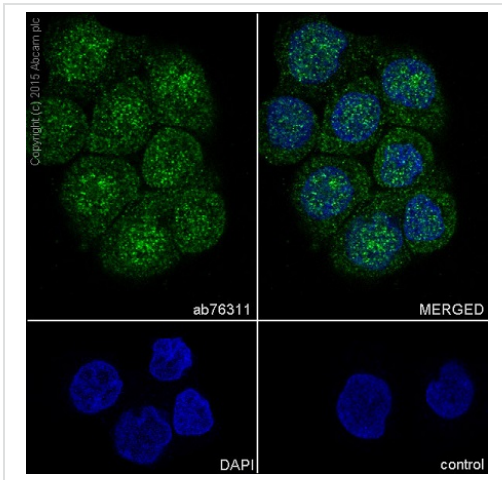
**Lane 3:** HeLa cell lysate (20 μg)

**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.

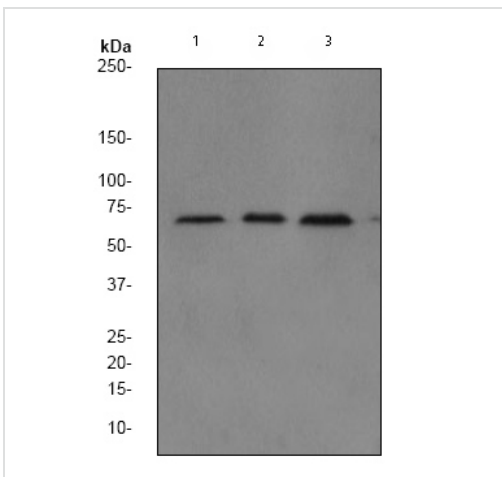
ab76311 was shown to specifically react with NF-κB p65 in wild-type HAP1 cells along with additional cross-reactive bands. No band was observed when NF-κB p65 knockout samples were used. Wild-type and NF-κB p65 knockout samples were subjected to SDS-PAGE. ab76311 (NF-κB p65) and ab8245 (loading control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

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

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