

Product datasheet

Anti-NF-kB p65 antibody [EP2161Y] ab76311

KO VALIDATED

Recombinant

RabMAb

★★★★★ 1 Abreviews 19 References 6 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, ICC/IF, Flow Cyt (Intra) Unsuitable for: IHC-P or IP
Species reactivity	Reacts with: Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, Daudi and Jurkat cell lysates. Flow Cyt (intra): HeLa and HT-29 cells. ICC: HT-29 cells
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p> <p>Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.</p>

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	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.5% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EP2161Y
Isotype	IgG

Applications

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

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.

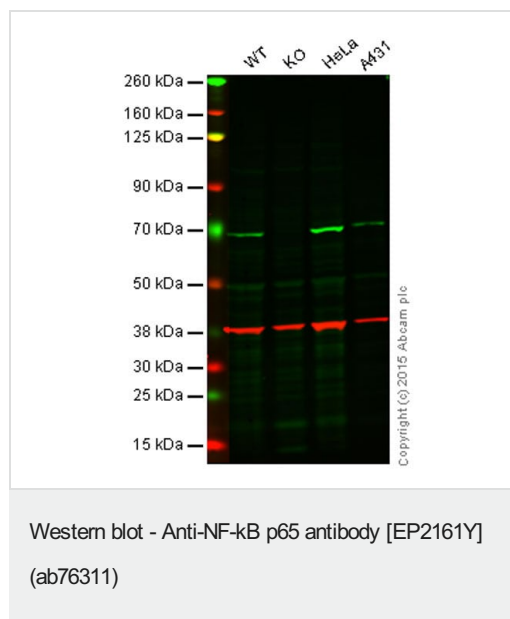
Phosphorylation at Ser-311 disrupts the interaction with EHMT1 and promotes transcription factor activity (By similarity). Phosphorylation on Ser-536 stimulates acetylation on Lys-310 and interaction with CBP; the phosphorylated and acetylated forms show enhanced transcriptional activity.

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)

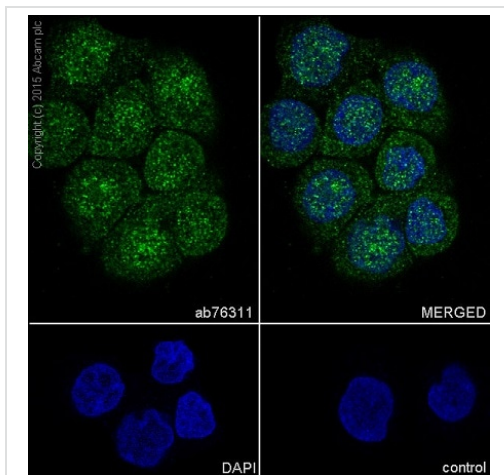
Lane 2: NF-kB p65 knockout HAP1 cell lysate (20 µg)

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.

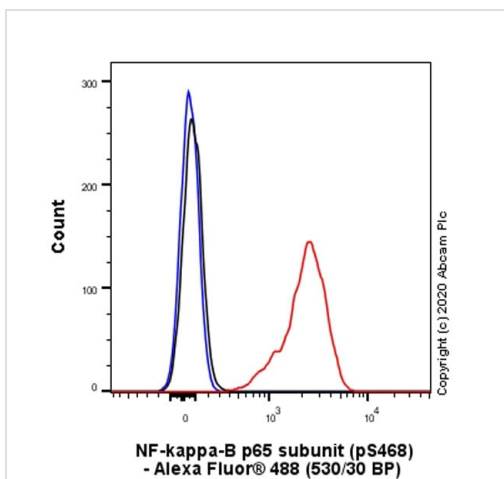
ab76311 was shown to specifically react with NF-kB p65 in wild-type HAP1 cells along with additional cross-reactive bands. No band was observed when NF-kB p65 knockout samples were used. Wild-type and NF-kB p65 knockout samples were subjected to SDS-PAGE. ab76311 (NF-kB p65) and **ab8245** (loading control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

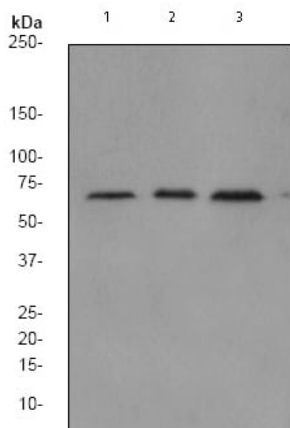
Immunocytochemistry/Immunofluorescence analysis HT-29 (human colorectal adenocarcinoma) labelling NF-kB p65 with purified ab76311 at 1/500. Cells were fixed with 4% PFA and permeabilized with 0.1% Triton X-100. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody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

Control: PBS only



Flow Cytometry (Intracellular) - Anti-NF-kB p65 antibody [EP2161Y] (ab76311)

Flow Cytometry analysis of HT-29 (Human colorectal adenocarcinoma epithelial cell) cells labeling NF-kB p65 with purified ab76311 at 1/1000 dilution (1 µg/mL) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



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

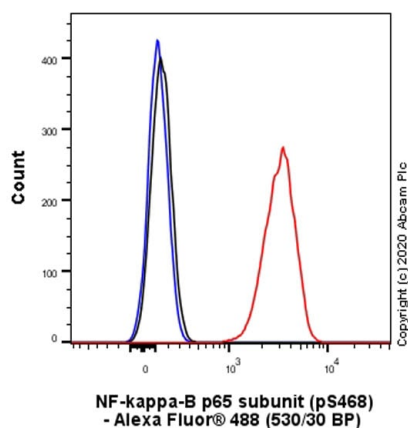
Lysates/proteins at 10 µg per lane.

Secondary

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

Predicted band size: 65 kDa

Observed band size: 70 kDa



Flow Cytometry (Intracellular) - Anti-NF-kB p65 antibody [EP2161Y] (ab76311)

Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling NF-kB p65 with purified ab76311 at 1/1000 dilution (1 µg/mL) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, [ab150077](#)) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).

Why choose a recombinant antibody?



Research with confidence
Consistent and reproducible results



Long-term and scalable supply
Recombinant technology



Success from the first experiment
Confirmed specificity



Ethical standards compliant
Animal-free production

Anti-NF- κ B p65 antibody [EP2161Y] (ab76311)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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