

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

敲除验证
重组
RabMAb

★★★★☆
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#### 概述

产品名称	Anti-NF-kB p65抗体[EP2161Y]
描述	兔单克隆抗体[EP2161Y] to NF-kB p65
宿主	Rabbit
特异性	ab76311 detects both phosphorylated and non-phosphorylated versions of human NF-kB p65.
经测试应用	<b>适用于:</b> WB, ICC/IF, Flow Cyt (Intra) <b>不适用于:</b> IHC-P or IP
种属反应性	<b>与反应:</b> Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: HeLa, Daudi and Jurkat cell lysates. Flow Cyt (intra): HeLa and HT-29 cells. ICC: HT-29 cells
常规说明	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> <li>- High batch-to-batch consistency and reproducibility</li> <li>- Improved sensitivity and specificity</li> <li>- Long-term security of supply</li> <li>- Animal-free production</li> </ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a>.</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>

#### 性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.5% BSA
纯度	Protein A purified

克隆	单克隆
克隆编号	EP2161Y
同种型	IgG

## 应用

**The Abpromise guarantee**      **Abpromise™承诺保证使用ab76311于以下的经测试应用**

“应用说明”部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
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. <b>ab172730</b> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

**应用说明** Is unsuitable for IHC-P or IP.

靶标

**功能**

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.

**序列相似性** Contains 1 RHD (Rel-like) domain.

**结构域** the 9aaTAD motif is a transactivation domain present in a large number of yeast and animal transcription factors.

翻译后修饰

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.

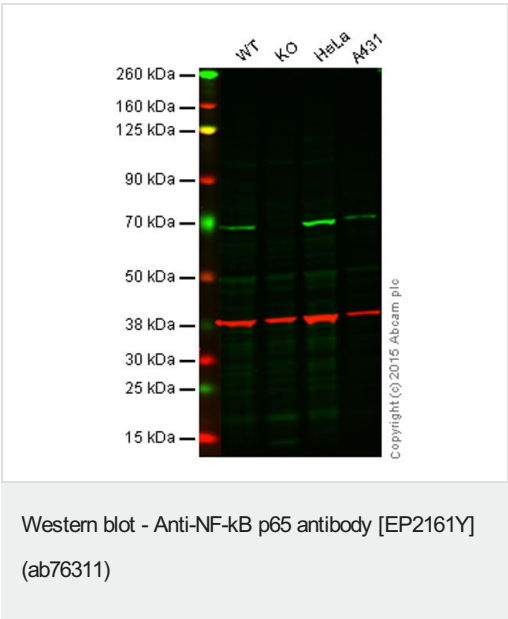
Phosphorylation at Ser-311 disrupts the interaction with EHMT1 and promotes transcription factor activity (By similarity). Phosphorylation on Ser-536 stimulates acetylation on Lys-310 and interaction with CBP; the phosphorylated and acetylated forms show enhanced transcriptional activity.

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'.

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.

细胞定位

图片



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

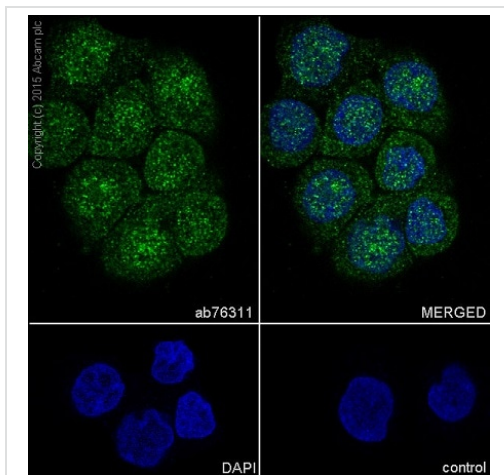
**Lane 2:** NF-κB p65 knockout HAP1 cell lysate (20 µg)

**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.

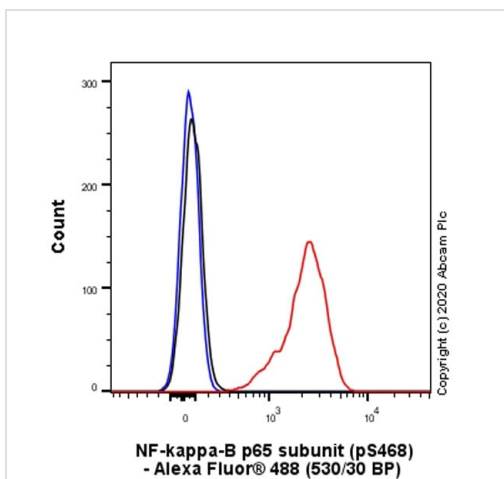
ab76311 was shown to specifically react with NF-κB p65 in wild-type HAP1 cells along with additional cross-reactive bands. No band was observed when NF-κB p65 knockout samples were used. Wild-type and NF-κB p65 knockout samples were subjected to SDS-PAGE. ab76311 (NF-κB p65) and **ab8245** (loading control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

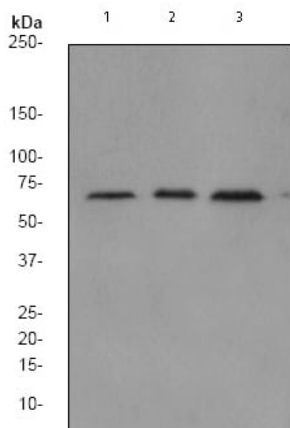
Immunocytochemistry/Immunofluorescence analysis HT-29 (human colorectal adenocarcinoma) labelling NF-kB p65 with purified ab76311 at 1/500. Cells were fixed with 4% PFA and permeabilized with 0.1% Triton X-100. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody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

Control: PBS only



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

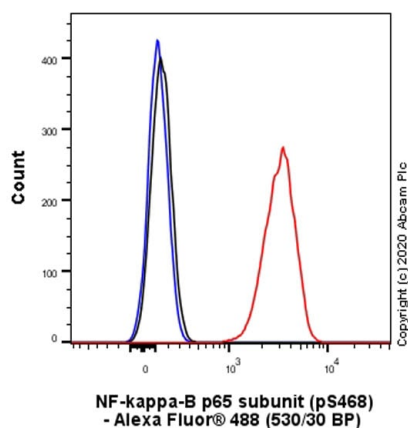
Lysates/proteins at 10 µg per lane.

#### 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- $\kappa$ B p65 antibody [EP2161Y] (ab76311)

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

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