abcam

ab76311

Anti-NF-kB p65 antibody [EP2161Y]

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EP2161Y
Purification technique	Affinity purification Protein A
Specificity	ab76311 detects both phosphorylated and non-phosphorylated versions of human NF-kB p65.
Concentration	0.306 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Not recommended

Species	Human
Dilution info	-

Notes	-

WB

Tested

Species	Human
Dilution info	1/1000 - 1/5000
Notes	-

IHC-P

Not recommended

Species	Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/100 - 1/500
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/1000
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Target data

See full target information RELA

Function	NF-kappa-B is a pleiotropic transcription factor present in almost all cell types and is the endpoint of a series of signal transduction events that are initiated by a vast array of stimuli related to 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. The heterodimeric RELA-NFKB1 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. The NF-kappa-B heterodimeric RELA-NFKB1 and RELA-REL complexes, for instance, function as transcriptional activators. 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. The inhibitory effect of I-kappa-B on NF-kappa-B through retention in the cytoplasm is exerted primarily through the interaction with RELA. RELA shows a weak DNA-binding site which could contribute directly to DNA binding in the NF-kappa-B complex. Besides its activity as a direct transcriptional activator, it is also able to modulate promoters accessibility to transcription factors and thereby indirectly regulate gene expression. Associates with chromatin at the NF-kappa-B promoter region via association with DDX1. Essential for cytokine gene expression in T-cells (PubMed:15790681). The NF-kappa-B homodimeric RELA-RELA complex appears to be involved in invasin-mediated activation of IL-8 expression. Key transcription factor regulating the IFN response during SARS-CoV-2 infection (PubMed:33440148).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Species reactivity
Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	NF-kB p65 also known as RelA is a significant component of the NF-kB protein complex. This complex usually involves a molecular weight for p65 of approximately 65 kDa. NF-kB p65 is mechanistically a transcription factor that regulates genes involved in inflammation cell survival and immune response. Expression of p65 is widely seen in various cell types including immune cells and epithelial cells suggesting its role in numerous physiological processes.
Biological function summary	NF-kB p65 acts as part of the larger NF-kB complex usually forming a heterodimer with other family members like p50. This complex translocates to the nucleus upon activation where it binds specific DNA sequences to regulate gene expression. The p65 subunit is essential for transactivating target genes involved in immune and inflammatory responses. Its regulation is important for proper cellular functioning especially in the maintenance of immune homeostasis and inflammatory response.
Pathways	NF-kB p65 participates in critical pathways like the NF-kB signaling and Toll-like receptor pathways. These pathways are fundamental for initiating immune responses and contribute to the regulation of apoptosis and cellular stress responses. In the context of these pathways the IKK complex plays a pivotal role in NF-kB activation leading to the phosphorylation and subsequent degradation of inhibitors that retain NF-kB p65 in the cytoplasm therefore allowing its movement to the nucleus.
Associated diseases and disorders	NF-kB p65 is implicated in conditions such as cancer and autoimmune diseases. Its dysregulation can lead to persistent activation of inflammatory pathways contributing to tumorigenesis and chronic inflammation. In the context of these diseases proteins such as the p50 subunit and the IKK complex are often involved reflecting the importance of tight regulation of NF-kB signaling in preventing pathological conditions.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

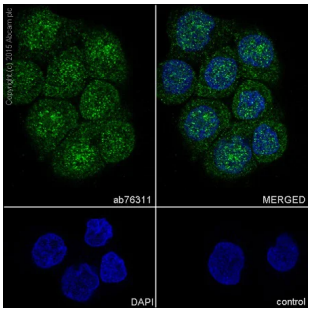
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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5 product images

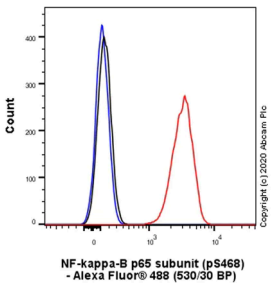


Immunocytochemistry/ Immunofluorescence - Anti-NF-kB p65 antibody [EP2161Y] (ab76311)

NF-kB p65 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-NF-kB p65 antibody

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

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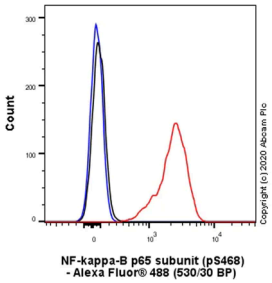


Flow Cytometry (Intracellular) - Anti-NF-kB p65 antibody [EP2161Y] (ab76311)

NF-kB p65 Flow Cytometry (Intracellular) staining using rabbit Anti-NF-kB p65 antibody

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,

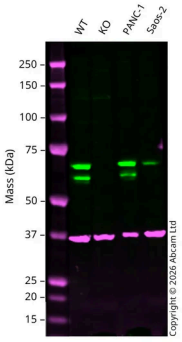
ab150077) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



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

NF-kB p65 Flow Cytometry (Intracellular) staining using rabbit Anti-NF-kB p65 antibody

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)

NF-kB p65 Western blot staining using rabbit Anti-NF-kB p65 antibody

Western blot: Anti-NF-kB p65 antibody [EP2161Y] ab76311 staining at 1/1000 dilution, shown in green; Mouse anti GAPDH ab8245 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 60 kDa in Wild-type MCF7 cell lysates with no signal observed at this size in RELA knockout MCF7 cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5% Milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit 800CW and Goat anti-Mouse 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-NF-kB p65 antibody [EP2161Y] (ab76311) at 1/1000 dilution

Lane 1:
Wild-type MCF7 cell lysates at 20 µg

Lane 2:
Western blot - Human RELA knockout MCF7 cell line (ab287740) at 40 µg

Lane 3:
PANC-1 cell lysates at 20 µg

Lane 4:
Saos-2 cell lysates at 20 µg

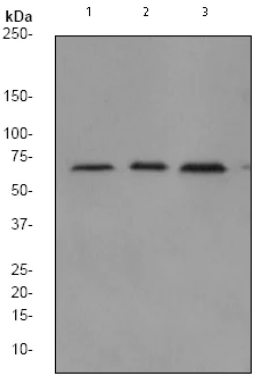
Secondary

All lanes:
Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 60 kDa

Observed band size: 60 kDa



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

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

Lane 1:
HeLa cell lysate at 10 µg

Lane 2:
Daudi cell lysate at 10 µg

Lane 3:
Jurkat cell lysate at 10 µg

Secondary

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

Predicted band size: 60 kDa

Observed band size: 70 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.