abcam

Product datasheet

Anti-NF-kB p65 antibody [EP2161Y] - BSA and Azide free ab246347

KO VALIDATED Recombinant RabMAb

1 References 5 Images

Overview

Product name	Anti-NF-kB p65 antibody [EP2161Y] - BSA and Azide free
Description	Rabbit monoclonal [EP2161Y] to NF-kB p65 - BSA and Azide free
Host species	Rabbit
Specificity	ab246347 detects both phosphorylated and non-phosphorylated versions of human NF-kB p65.
Tested applications	**Suitable for:** ICC/IF, WB, 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	HeLa, Daudi and Jurkat cell lysates. Flow Cyt (intra): HT-29 and HeLa cells
General notes	ab246347 is the carrier-free version of **ab76311**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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 production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit

monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

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

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP2161Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab246347 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
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 70 kDa (predicted molecular weight: 65 kDa).
Flow Cyt (Intra)		Use at an assay dependent concentration.

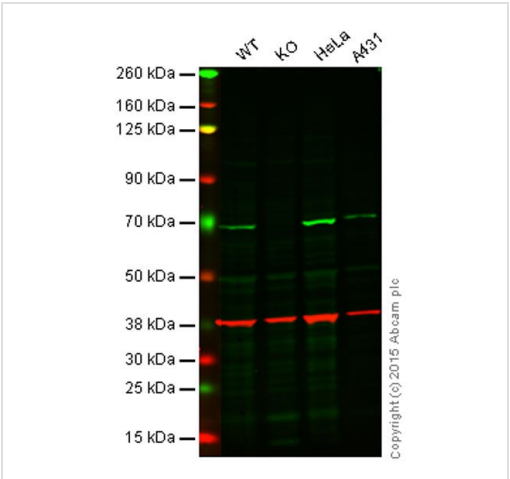
Application notes Is unsuitable for IHC-P or IP.

Target

Function NF-kappa-B is a pleiotropic transcription factor which is present in almost all cell types and is involved in many biological processed 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 invasin-mediated activation of IL-8 expression. The inhibitory effect of I-kappa-B upon NF-kappa-B 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



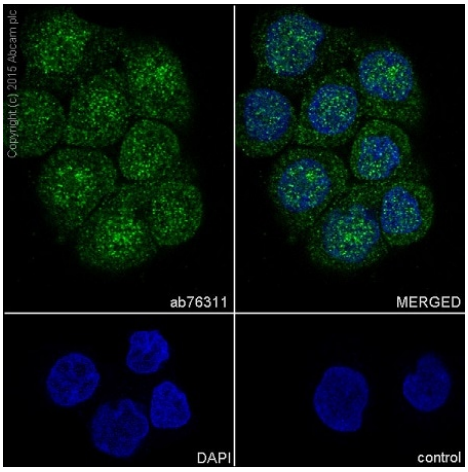
Western blot - Anti-NF-kB p65 antibody [EP2161Y] - BSA and Azide free (ab246347)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab76311**).

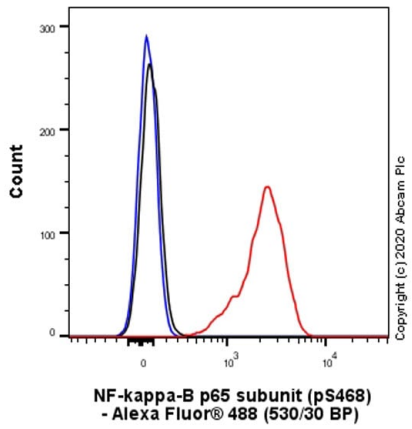


Immunocytochemistry/ Immunofluorescence - Anti-NF-kB p65 antibody [EP2161Y] - BSA and Azide free (ab246347)

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

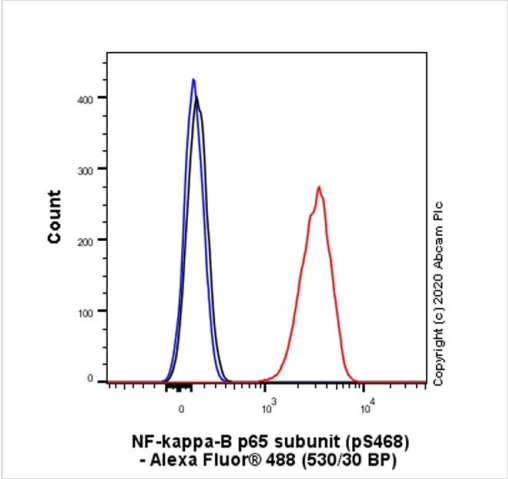
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab76311**).



Flow Cytometry (Intracellular) - Anti-NF-kB p65 antibody [EP2161Y] - BSA and Azide free (ab246347)

This data was developed using **ab76311**, the same antibody clone in a different buffer formulation.

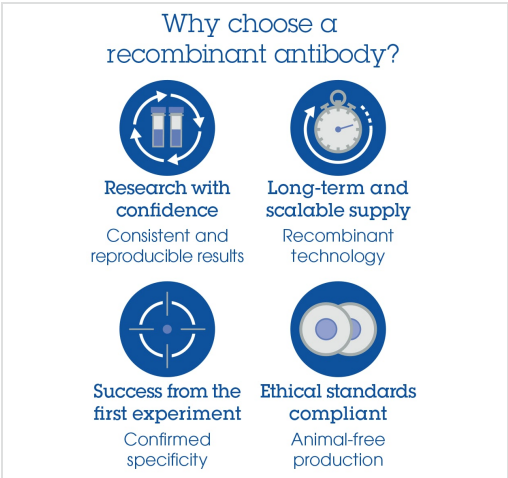
Flow Cytometry analysis of HT-29 (Human colorectal adenocarcinoma epithelial cell) cells labeling NF-kB p65 with purified **ab76311** at 1/1000 dilution (1 µg/mL) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) 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).



Flow Cytometry (Intracellular) - Anti-NF-kB p65 antibody [EP2161Y] - BSA and Azide free (ab246347)

This data was developed using **ab76311**, the same antibody clone in a different buffer formulation.

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).



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