abcam

Product datasheet

Anti-NF-kB p65 antibody [E379] - BSA and Azide free ab207297

敲除验证 重组 RabMAb

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概述

产品名称	Anti-NF-kB p65抗体[E379] - BSA and Azide free
描述	兔单克隆抗体[E379] to NF-kB p65 - BSA and Azide free
宿主	Rabbit
特异性	This antibody recognises NF-kB p65. For WB, this antibody is unsuitable for detecting NF-KB p65 in mouse tissue lysates. The expression of NF-KB p65 is increased by lipopolysaccharides treatment reported by PMID: 18036230. Although some papers support the expression of NF-κB p65 in mouse tissue (PMID: 21479220 and 20008488), This antibody cannot detect band of interest in these mouse tissue.
经测试应用	**适用于:** ICC/IF, IP, WB, IHC-P **不适用于:** Flow Cyt
种属反应性	**与反应:** Mouse, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: Wild-type HAP1 cell lysate. HeLa, MEF, RAW 264.7 and A431 cell lysate; Human fetal brain, kidney and lung tissue lysates. IHC-P: Human breast carcinoma and colon carcinoma tissue. Mouse colon tissue. Mouse spleen, human tonsil and human prostatic hyperplasia tissues. ICC/IF: HeLa and NIH/3T3 cells. IP: NF-kB p65 IP in HeLa whole cell lysate (**ab150035**).
常规说明	ab207297 is the carrier-free version of **ab32536**. 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**.

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

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C. Do Not Freeze.
存储溶液	pH: 7.20 Constituent: PBS
无载体	**是**
纯度	Protein A purified
克隆	单克隆
克隆编号	E379
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab207297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
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 65 kDa (predicted molecular weight: 65 kDa).
IHC-P	★★★☆☆ (1)	Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

应用说明 Is unsuitable for Flow Cyt.

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 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.

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.

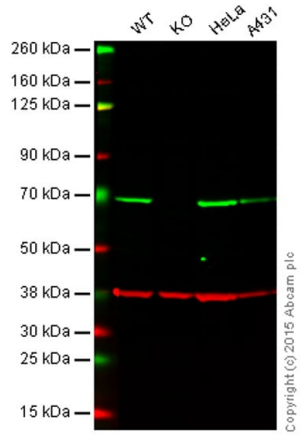
翻译后修饰

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'.

细胞定位

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.

图片



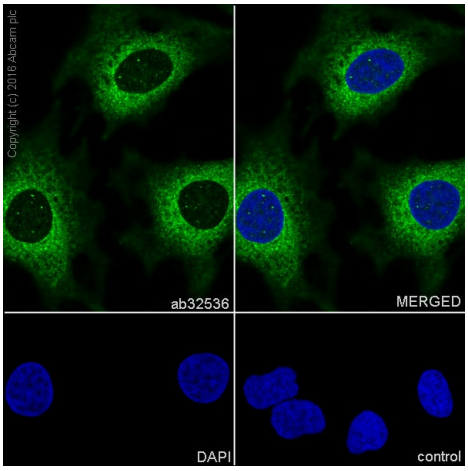
Western blot - Anti-NF-kB p65 antibody [E379] - BSA and Azide free (ab207297)

This WB data was generated using the same anti-NF-kB p65 antibody clone, E379, in a different buffer formulation (cat# **ab32536**).

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 - **ab32536** observed at 70 kDa. Red - **ab8245** loading control, observed at 37 kDa.

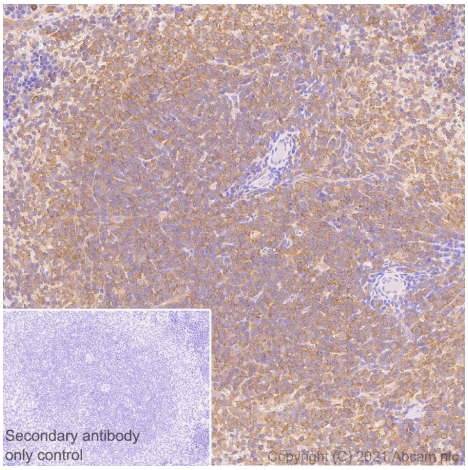
ab32536 was shown to specifically react with NF-kB p65 in wild-type HAP1 cells. No band was observed when NF-kB p65 knockout samples were used. Wild-type and NF-kB p65 knockout samples were subjected to SDS-PAGE. **ab32536** (NF-kB p65) and **ab8245** (loading control to GAPDH) were diluted to 1/50 000 and 1/2000 respectively 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 1/10 000 dilution for 1 h at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-NF-kB p65 antibody [E379] - BSA and Azide free (ab207297)

Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling NF-kB p65 with purified **ab32536** at 1:100 dilution. Cells were fixed in 100% Methanol. **ab150077** Goat anti rabbit IgG(Alexa Fluor® 488) was used as the secondary antibody at 1:1000 dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32536**).



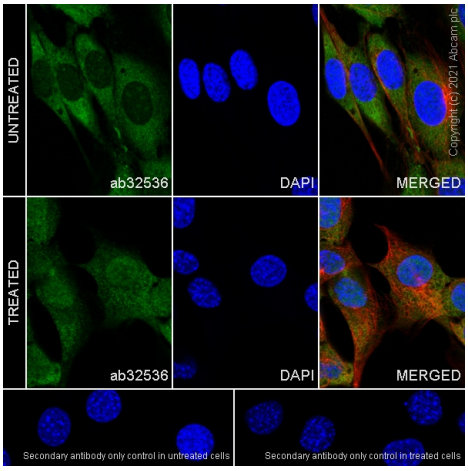
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NF-kB p65 antibody [E379] - BSA and Azide free (ab207297)

This data was generated using the same anti-NF-kB p65 antibody clone, E379, in a different buffer formulation (**ab32536**).

Immunohistochemical analysis of paraffin-embedded Mouse spleen tissue labeling NF-kB p65 with **ab32536** at 1/5000 (0.098 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Cytoplasmic staining on mouse spleen. The section was incubated with **ab32536** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins

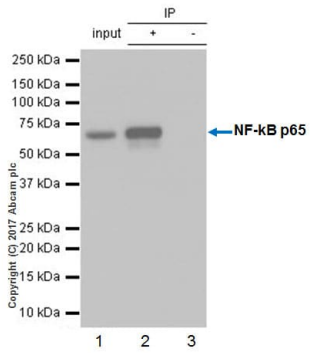


Immunocytochemistry/ Immunofluorescence - Anti-NF-kB p65 antibody [E379] - BSA and Azide free (ab207297)

This data was generated using the same anti-NF-kB p65 antibody clone, E379, in a different buffer formulation (**ab32536**).

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized NIH/3T3 cells labelling NF-kB p65 with **ab32536** at 1/100 (4.89 ug/ml) dilution, followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (Green). Confocal image showing the signal translocated from the cytoplasm into the nucleus in NIH/3T3 cells after the treatment with TNF-alpha (50 ng/ml) for 20 min. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution in treated (right) and untreated cells (left).



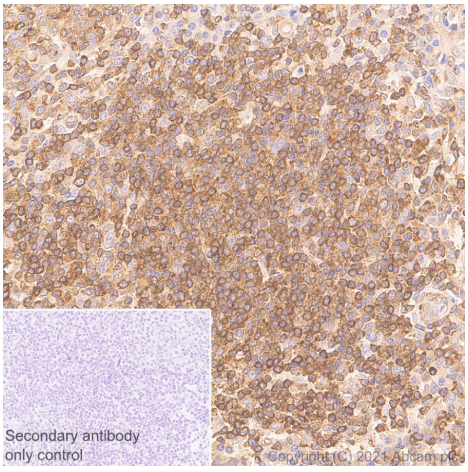
Immunoprecipitation - Anti-NF-kB p65 antibody [E379] - BSA and Azide free (ab207297)

ab32536 (purified) at 1:30 dilution (2µg) immunoprecipitating NF-kB p65 in HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate.

Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10µg
Lane 2 (+): **ab32536** & HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of **ab32536** in HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1:1000 dilution.
Blocking and diluting buffer: 5% NFDM/TBST.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32536**).



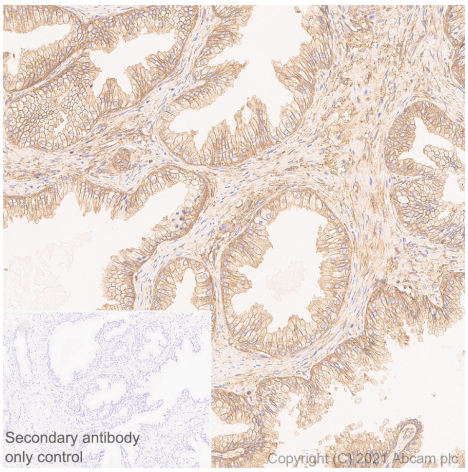
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NF-kB p65 antibody [E379] - BSA and Azide free (ab207297)

This data was generated using the same anti-NF-kB p65 antibody clone, E379, in a different buffer formulation (**ab32536**).

Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labeling NF-kB p65 with **ab32536** at 1/5000 (0.098 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Cytoplasmic staining on human tonsil. The section was incubated with **ab32536** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins



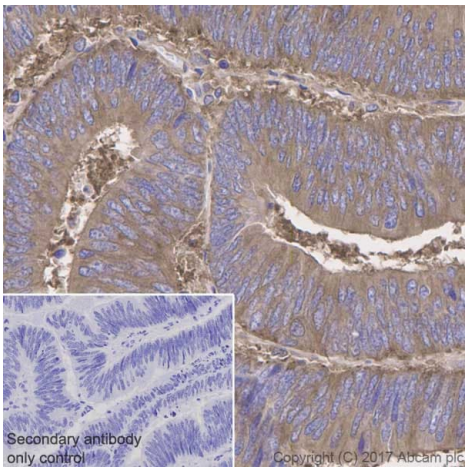
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NF-kB p65 antibody [E379] - BSA and Azide free (ab207297)

This data was generated using the same anti-NF-kB p65 antibody clone, E379, in a different buffer formulation (**ab32536**).

Immunohistochemical analysis of paraffin-embedded Human prostatic hyperplasia tissue labeling NF-kB p65 with **ab32536** at 1/5000 (0.098 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Cytoplasmic staining on human prostatic hyperplasia. The section was incubated with **ab32536** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

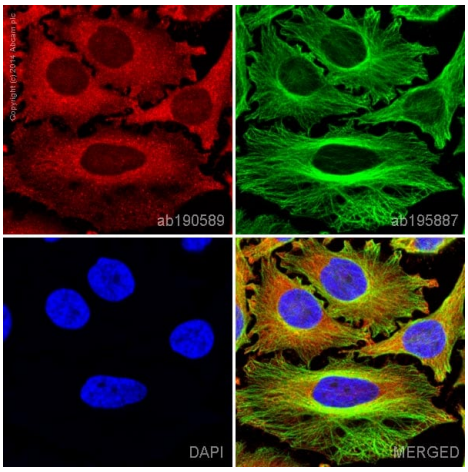
Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NF-kB p65 antibody [E379] - BSA and Azide free (ab207297)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human colon carcinoma tissue sections labeling NF-kB p65 with Purified **ab32536** at 1:2000 dilution (0.2 µg/ml). Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32536**).



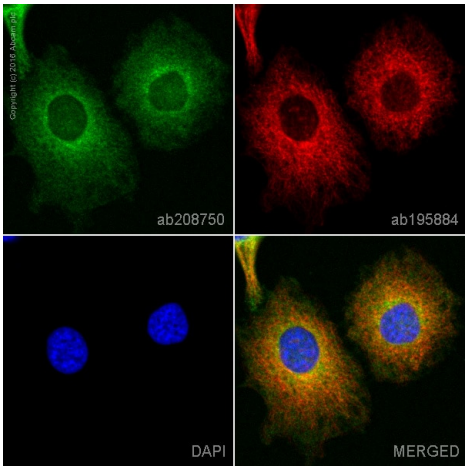
Immunocytochemistry/ Immunofluorescence - Anti-NF-kB p65 antibody [E379] - BSA and Azide free (ab207297)

Clone E379 (ab207297) has been successfully conjugated by Abcam. This image was generated using Anti-NF-kB p65 antibody [E379] (Alexa Fluor® 647). Please refer to **ab190589** for protocol details.

ab190589 staining NF-kB p65 in HeLa cells. The cells were fixed with 4% formaldehyde (10 min), permeabilized in 0.1% Triton X-100 for 5 minutes and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab190589** at a working dilution of 1 in 100 (shown in red) and **ab195887**, Mouse monoclonal [DM1A] to alpha Tubulin (Alexa Fluor® 488, shown in green) at 2µg/ml overnight at +4°C. Nuclear DNA was labelled in blue with DAPI.

This product also gave a positive signal in 100% methanol (5 min) fixed HeLa cells under the same testing conditions.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



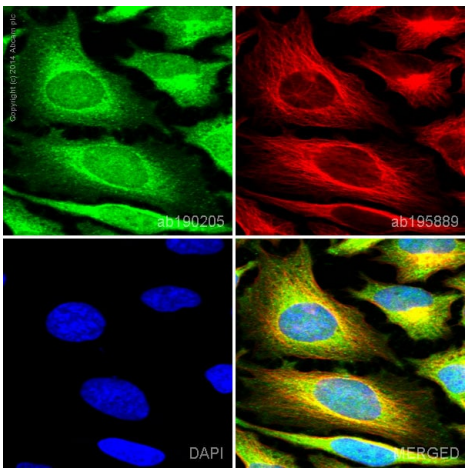
Immunocytochemistry/ Immunofluorescence - Anti-NF-kB p65 antibody [E379] - BSA and Azide free (ab207297)

Clone E379 (ab207297) has been successfully conjugated by Abcam. This image was generated using Anti-NF-kB p65 antibody [E379] (PE). Please refer to **ab208750** for protocol details.

ab208750 staining NF-kB p65 in HeLa cells. The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab208750** at 1/500 dilution (Pseudocolored in green) and **ab195884**, Rat monoclonal to Tubulin (Alexa Fluor® 647), at 1/250 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

This product also gave a positive signal under the same testing conditions in HeLa cells fixed with 100% methanol (5min).



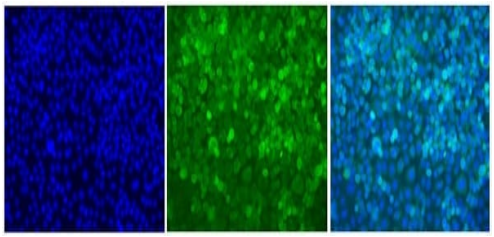
Immunocytochemistry/ Immunofluorescence - Anti-NF-kB p65 antibody [E379] - BSA and Azide free (ab207297)

Clone E379 (ab207297) has been successfully conjugated by Abcam. This image was generated using Anti-NF-kB p65 antibody [E379] (Alexa Fluor® 488). Please refer to **ab190205** for protocol details.

ab190205 staining NF-kB p65 in HeLa cells. The cells were fixed with 100% methanol (5 min), permeabilized in 0.1% Triton X-100 for 5 minutes and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with **ab190205** at a working dilution of 1 in 50 (shown in green) and **ab195889**, Mouse monoclonal [DM1A] to alpha Tubulin (Alexa Fluor® 594, shown in red) at a dilution of 1 in 250 overnight at +4°C. Nuclear DNA was labelled in blue with DAPI.

This product also gave a positive signal in 4% formaldehyde (10 min) fixed HeLa cells under the same testing conditions.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

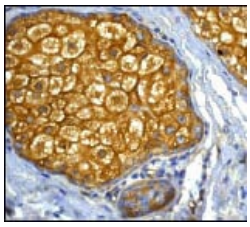


Immunocytochemistry/ Immunofluorescence - Anti-NF-kB p65 antibody [E379] - BSA and Azide free (ab207297)

Image from Ali Ahmed Atef Ahmed et al. PLoS ONE 11.4 (2016): e0154278. Fig 8. doi: 10.1371/journal.pone.0154278.

Immunocytochemistry/ Immunofluorescence analysis of human cancer cells labeling NF-kB p65 with unpurified **ab32536**.
Briefly, the tested cells were seeded on coverslips treated with HCl and ethanol, and autoclaved prior to use. Immunostaining of the p65 subunit of NF-κB was done by permeabilizing the cells with Triton X-10, then by treating the cells with anti-NF-κB p65 rabbit monoclonal primary antibody [E379] (**ab32536**), followed by Alexa Fluor® 488 Donkey anti-rabbit IgG secondary antibody. Nuclei of cells were stained with DAPI. Images were acquired using fluorescence microscope.

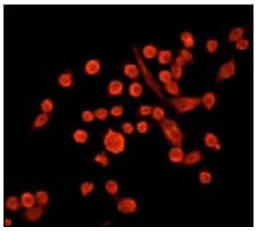
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32536**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NF-kB p65 antibody [E379] - BSA and Azide free (ab207297)

Immunohistochemical analysis of paraffin-embedded human Breast carcinoma using unpurified anti-NF-kB p65 Rabbit Monoclonal Antibody

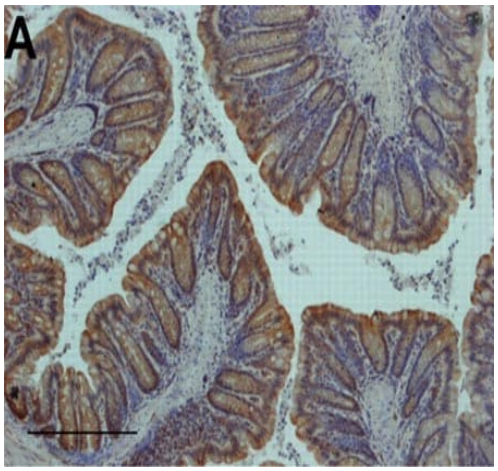
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32536**).



Immunocytochemistry/ Immunofluorescence - Anti-NF-kB p65 antibody [E379] - BSA and Azide free (ab207297)

Immunofluorescent staining of HeLa cells using anti-NF-kB p65 Rabbit Monoclonal Antibody (unpurified **ab32536**)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32536**).

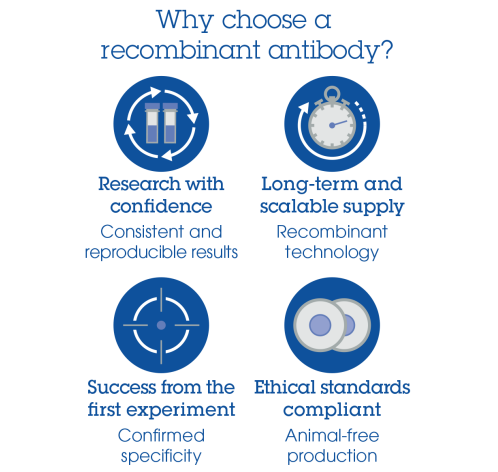


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NF-kB p65 antibody [E379] - BSA and Azide free (ab207297)

This IHC data was generated using the same anti-NF-kB p65 antibody clone, E379, in a different buffer formulation (cat# **ab32536**).

Immunohistochemical analysis of colon sections from mice, staining NF-kB p65 with **ab32536**.

Antigen retrieval was performed by microwave heating in citrate buffer, pH 6. Sections were incubated overnight with primary antibody (1/250) and staining was detected using **ab80437** EXPOSE Rabbit specific HRP/DAB detection IHC kit.



Anti-NF-kB p65 antibody [E379] - BSA and Azide free (ab207297)

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