abcam

Product datasheet

Anti-NF-kB p65 antibody [E379] ab32536

KO VALIDATED Recombinant RabMAb

15 Abreviews 338 References 21 Images

Overview

Product name	Anti-NF-kB p65 antibody [E379]
Description	Rabbit monoclonal [E379] to NF-kB p65
Host species	Rabbit
Specificity	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.
Tested applications	**Suitable for:** WB, IHC-P, ICC/IF, IP **Unsuitable for:** Flow Cyt
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Synthetic peptide within Human NF-kB p65 aa 500 to the C-terminus (C terminal). The exact sequence is proprietary. Database link: **Q04206**
Positive control	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**).
General notes	Abcam recommended secondaries - Goat Anti-Rabbit HRP (**ab205718**) and Goat Anti-Rabbit Alexa Fluor® 488 (**ab150077**). See other **anti-rabbit secondary antibodies** that can be used with this 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 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.

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: PBS, 40% Glycerol (glycerin, glycerine), 0.21% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	E379
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32536 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	★★★★☆ **(12)**	1/1000 - 1/100000. Detects a band of approximately 65 kDa (predicted molecular weight: 65 kDa). This antibody might not detect target band in mouse tissues.
IHC-P		1/2000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF	★★★★☆ **(2)**	1/100. **For unpurified use at 1/250 -1/500.**
IP		1/30. **For unpurified use at 1/200.** We do not guarantee IP for mouse.

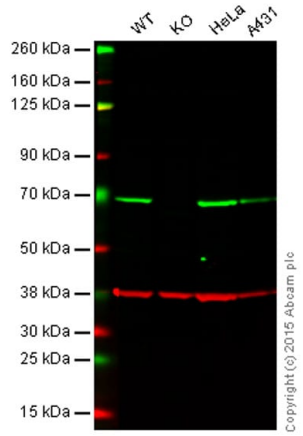
Application notes Is unsuitable for Flow Cyt.

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

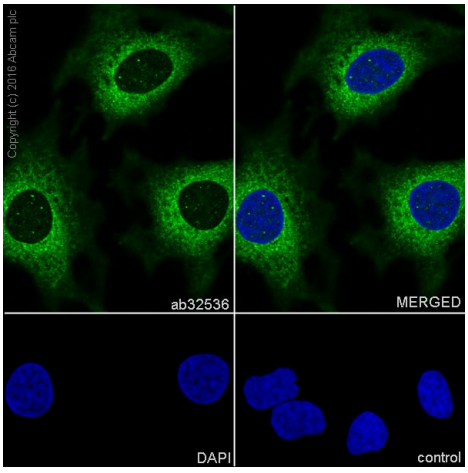


Western blot - Anti-NF-kB p65 antibody [E379] (ab32536)

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.

Unpurified 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.

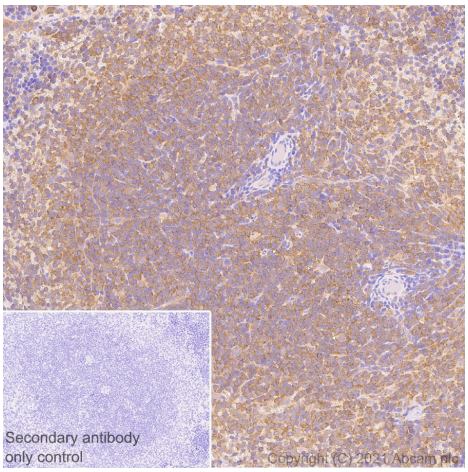


Immunocytochemistry/ Immunofluorescence - Anti-NF-kB p65 antibody [E379] (ab32536)

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.

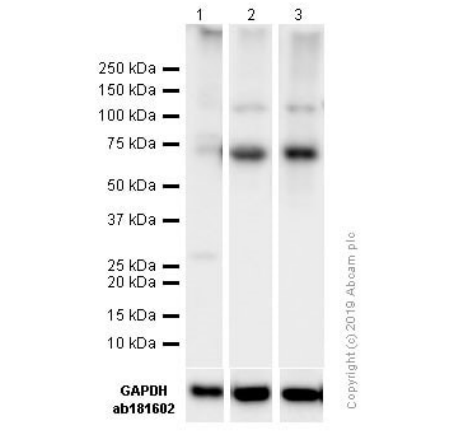


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NF-kB p65 antibody [E379] (ab32536)

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 (pH6.0, epitope retrieval solution 1) for 20 mins



Western blot - Anti-NF-kB p65 antibody [E379] (ab32536)

All lanes : Anti-NF-kB p65 antibody [E379] (ab32536) at 1/1000 dilution

Lane 1 : Human fetal brain lysates
Lane 2 : Human fetal kidney lysates
Lane 3 : Human fetal lung lysates

Lysates/proteins at 20 µg per lane.

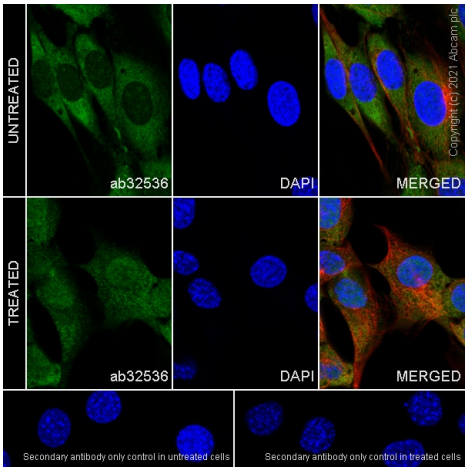
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 65 kDa
Observed band size: 65 kDa

Blocking/Diluting buffer and concentration: 5% NFDM/TBST

Exposure time: 37 second for Lane1 and 3 seconds for Lanes 2 and 3

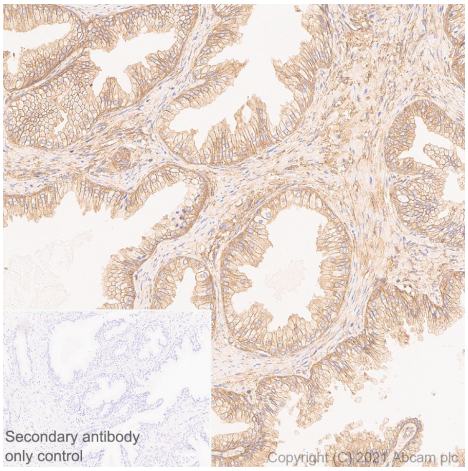
Normal brain might express low level of p65 (PMID: 21479220)



Immunocytochemistry/ Immunofluorescence - Anti-NF-kB p65 antibody [E379] (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).

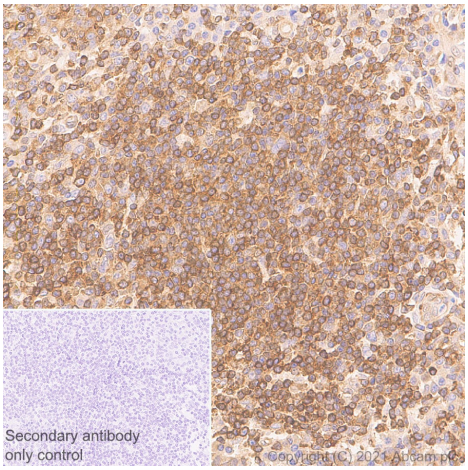


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NF-kB p65 antibody [E379] (ab32536)

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

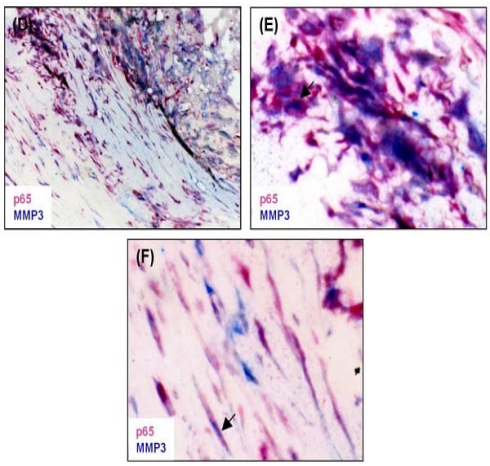


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NF-kB p65 antibody [E379] (ab32536)

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] (ab32536)

Souslova et al PLoS One. 2010 Mar 25;5(3):e9902. doi: 10.1371/journal.pone.0009902. Fig 2. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

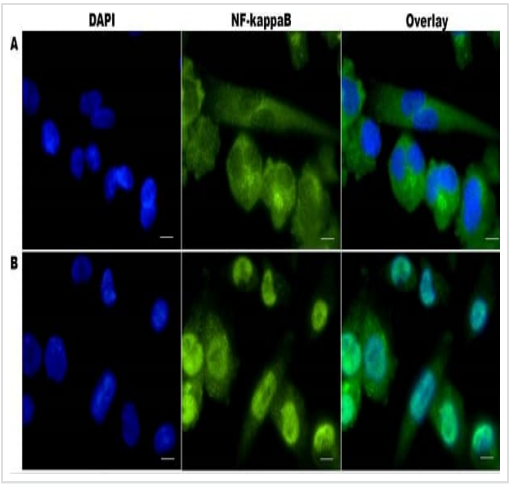
MMP3 and NFκB are co-localized in human atherosclerotic plaques.

Sections (4 μm) of paraffin embedded tissue blocks of atherosclerotic plaques were subjected to double immunostaining of MMP3 and p50 (A, B, and C) or MMP3 and p65 (**D, E and F, shown**), using rabbit polyclonal NFκB p50 and MMP3 antibodies (Abcam) and ab32536, respectively, and goat anti-rabbit secondary antibodies.

The double immunostaining was visualized with Liquid Permanent Red chromogen and Vector Blue chromogen. Pink colour indicates expression of p50 (A, B and C) or p65 (D, E and F). Blue colour indicates expression of MMP3 (A, B, C, D, E and F.

Arrow indicates cell co-expressing MMP3 with p50 (B and C) or with p65 (E and F).

200× magnification in A and D; 400× magnification in B, C, E and F.



Immunocytochemistry/ Immunofluorescence - Anti-NF-kB p65 antibody [E379] (ab32536)

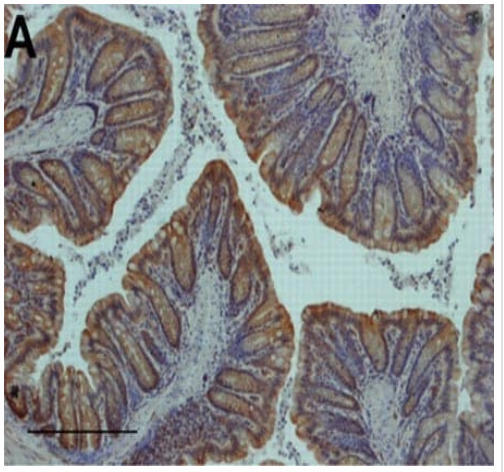
Fazalul Rahiman et al PLoS One. 2016 Apr 7;11(4):e0153005. doi: 10.1371/journal.pone.0153005. eCollection 2016. Fig 1. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

NF-κB/p65 nuclear localisation in differentiated THP-1 (Human monocytic leukemia cell line) cells.

Cells were fixed, permeabilized, stained with ab32536 and visualised with Alexa Fluor® 555 goat anti-rabbit IgG (green). The nuclei were counterstained with DAPI (blue). Microscopy images **A.** Inactive NF-κB/p65 proteins localization in the cytoplasm of the non-stimulated THP-1 cells (top row) and **B.** Translocated NF-κB/p65 proteins into the nuclei of THP-1 cells following LPS stimulation (bottom row).

Images were acquired for each fluorescence channel, using suitable TRITC and DAPI filters and a 63× objective.

Scale bar: 10µm

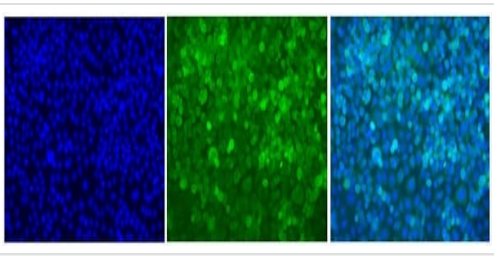


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NF-kB p65 antibody [E379] (ab32536)

Image from Bel S et al., J Biol Chem. 2012 Jul 20;287(30):25631-9. Epub 2012 May 2. Fig 8.; doi: 10.1074/jbc.M112.364786; July 20, 2012, The Journal of Biological Chemistry, 287, 25631-25639.

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



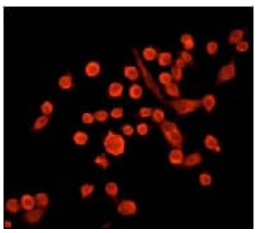
Immunocytochemistry/ Immunofluorescence - Anti-NF-kB p65 antibody [E379] (ab32536)

Immunocytochemistry/ Immunofluorescence analysis of human cancer cells labeling NF-kB p65 with unpurified ab32536 (Middle panel).

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 (Left panel).

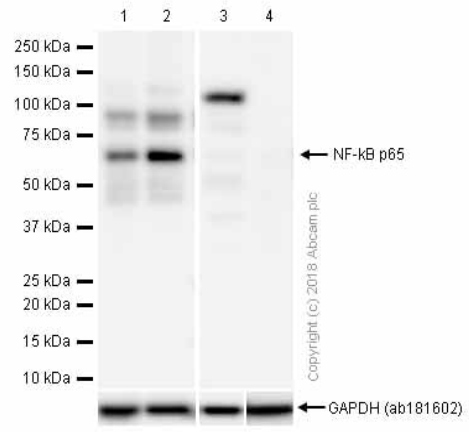
Merge (Right panel).

Images were acquired using fluorescence microscope.



Immunocytochemistry/ Immunofluorescence - Anti-NF-kB p65 antibody [E379] (ab32536)

Immunofluorescent staining of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells using unpurified ab32536.



Western blot - Anti-NF-kB p65 antibody [E379] (ab32536)

All lanes : Anti-NF-kB p65 antibody [E379] (ab32536) at 1000 µg

Lane 1 : Raw264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysates
Lane 2 : Raw264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) treated with 1ug/ml lipopolysaccharides for 6 hours whole cell lysates
Lane 3 : Mouse brain lysates
Lane 4 : Mouse spleen lysates

Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

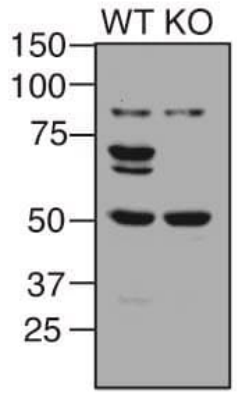
Predicted band size: 65 kDa

Exposure time: 3 seconds

This antibody is unsuitable for detecting 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), ab32536 cannot detect band of interest in these mouse tissue.

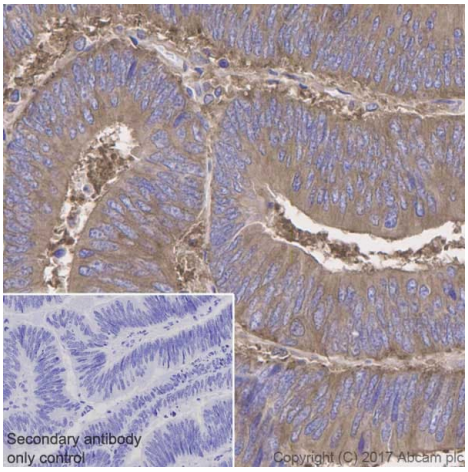


Western blot - Anti-NF-kB p65 antibody [E379] (ab32536)

Image courtesy of an anonymous Abreview

Anti-NF-kB p65 [E379] antibody (unpurified ab32536) reactivity with reduced wild type (WT) and p65 knockout (KO) mouse embryonic fibroblast (MEF) lysate.

After SDS-PAGE, membranes were blocked in 5% milk in TBS + 0.1% Tween for 1 hour at 25°C before incubation with unpurified ab32536 (1:1,000 dilution in 5% milk TBS + 0.1% Tween) for 16 hours at 4ºC. Blots was then incubated with an anti-Rabbit HRP-conjugated secondary antibody before developing with ECL.

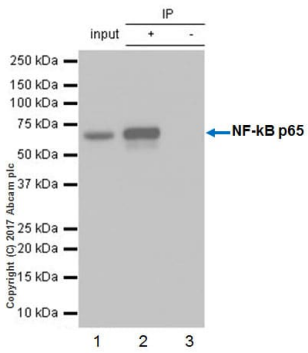


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NF-kB p65 antibody [E379] (ab32536)

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.

PBS instead of the primary antibody was used as the negative control.

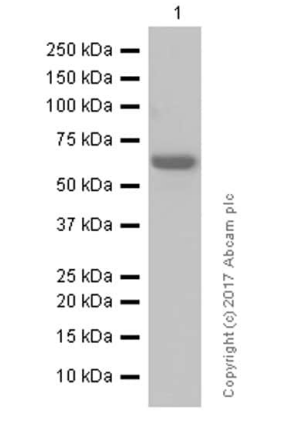


Immunoprecipitation - Anti-NF-kB p65 antibody [E379] (ab32536)

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

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



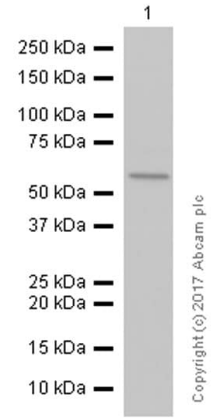
Western blot - Anti-NF-kB p65 antibody [E379] (ab32536)

Anti-NF-kB p65 antibody [E379] (ab32536) at 1/5000 dilution (purified) + HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates at 15 µg

Secondary
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 65 kDa

Blocking and diluting buffer: 5% NFDM/TBST.



Western blot - Anti-NF-kB p65 antibody [E379] (ab32536)

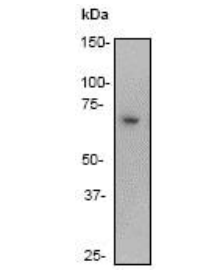
Anti-NF-kB p65 antibody [E379] (ab32536) at 1/5000 dilution (purified) + MEF (Mouse embryonic fibroblast (immortalized)) whole cell lysates at 15 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 65 kDa

Blocking and diluting buffer: 5% NFDM/TBST.

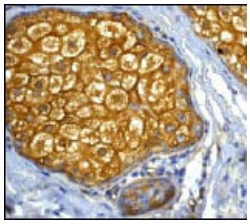


Western blot - Anti-NF-kB p65 antibody [E379] (ab32536)

Anti-NF-kB p65 antibody [E379] (ab32536) at 1/100000 dilution (unpurified) + HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate

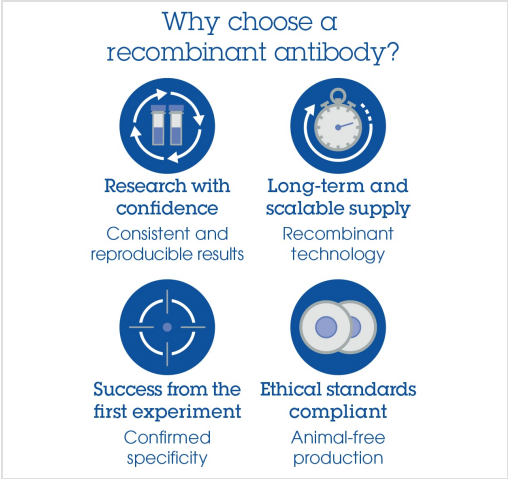
Predicted band size: 65 kDa

Observed band size: 65 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NF-kB p65 antibody [E379] (ab32536)

Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using unpurified ab32536.



Anti-NF-kB p65 antibody [E379] (ab32536)

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

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors