

Product datasheet

Anti-NF-kB p65 (phospho S536) antibody [EP2294Y] ab76302

Recombinant RabMAb

★★★★★ 7 Abreviews 36 References 5 Images

Overview

Product name	Anti-NF-kB p65 (phospho S536) antibody [EP2294Y]
Description	Rabbit monoclonal [EP2294Y] to NF-kB p65 (phospho S536)
Host species	Rabbit
Tested applications	Suitable for: Dot blot, WB, IP Unsuitable for: Flow Cyt, ICC/IF or IHC-P
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide (the amino acid sequence is considered to be commercially sensitive) within Human NF-kB p65 (phospho S536). The exact sequence is proprietary. (Peptide available as ab202905)
Positive control	WB: HeLa whole cell lysate treated with Calyculin A + TNF-alpha. C6 and NIH/3T3 treated with 100nM Calyculin A for 30 minutes whole cell lysate . IP: Daudi cell lysate treated with Calyculin A + TNF-alpha.
General notes	Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

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

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

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: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP2294Y
Isotype	IgG

Applications

Our [Abpromise guarantee](#) covers the use of **ab76302** 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
Dot blot		1/1000.
WB	★★★★☆	1/1000. Predicted molecular weight: 65 kDa.
IP		1/20 - 1/30.

Application notes Is unsuitable for Flow Cyt, ICC/IF or IHC-P.

Target

Function

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.

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.

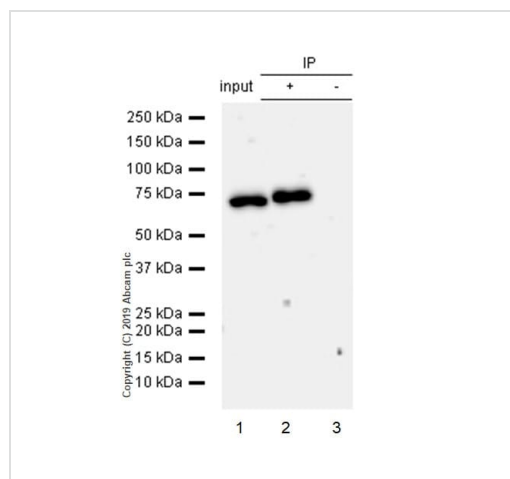
Phosphorylation at Ser-311 disrupts the interaction with EHMT1 and promotes transcription factor activity (By similarity). Phosphorylation on Ser-536 stimulates acetylation on Lys-310 and interaction with CBP; the phosphorylated and acetylated forms show enhanced transcriptional activity.

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



Immunoprecipitation - Anti-NF-kB p65 (phospho S536) antibody [EP2294Y] (ab76302)

ab76302 at 1/30 immunoprecipitating NF-kB p65 (phospho S536) in Daudi whole cell lysate treated with calyculin A and tumor necrosis factor-alpha.

Lane 1 (input): Daudi whole cell lysate treated with calyculin A and tumor necrosis factor-alpha (10µg)

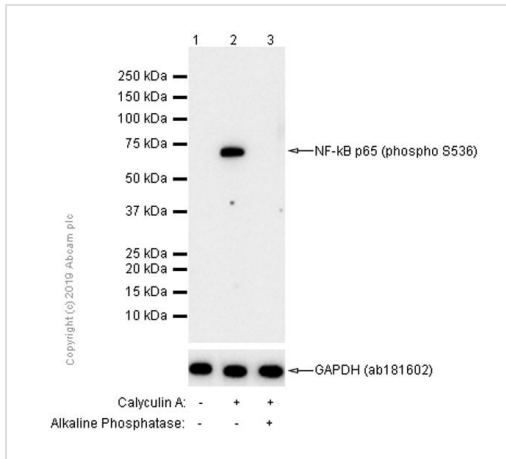
Lane 2 (+): ab76302 + Daudi whole cell lysate treated with calyculin A and tumor necrosis factor-alpha.

Lane 3 (-): Rabbit monoclonal IgG ([ab172730](#)) instead of ab76302 in Daudi whole cell lysate treated with calyculin A and tumor necrosis factor-alpha.

For western blotting, ab76302 at 1/500 dilution (0.95 µg/ml) and VeriBlot for IP Detection Reagent (HRP)([ab131366](#)) at 1/1000 dilution were used.

Blocking buffer and concentration: 5% NFDm/TBST.

Diluting buffer and concentration: 5% NFDm /TBST.



Western blot - Anti-NF-kB p65 (phospho S536) antibody [EP2294Y] (ab76302)

All lanes : Anti-NF-kB p65 (phospho S536) antibody [EP2294Y] (ab76302) at 1/1000 dilution

Lane 1 : C6 (Rat glioma tumor glioma cell) whole cell lysate

Lane 2 : C6 (Rat glioma tumor glioma cell) treated with 100nM Calyculin A for 30 minutes whole cell lysate

Lane 3 : C6 (Rat glioma tumor glioma cell) treated with 100nM Calyculin A for 30 minutes, then the membrane treated with Alkaline Phosphatase for 1 hour

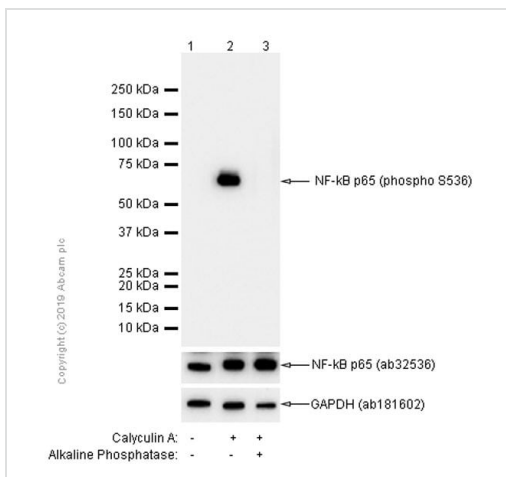
Lysates/proteins at 15 µg per lane.

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



Western blot - Anti-NF-kB p65 (phospho S536) antibody [EP2294Y] (ab76302)

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/1000 dilution

Lane 1 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate

Lane 2 : NIH/3T3 (Mouse embryonic fibroblast) treated with 100nM Calyculin A for 30 minutes whole cell lysate

Lane 3 : NIH/3T3 (Mouse embryonic fibroblast) treated with 100nM Calyculin A for 30 minutes, then the membrane treated with Alkaline Phosphatase for 1 hour

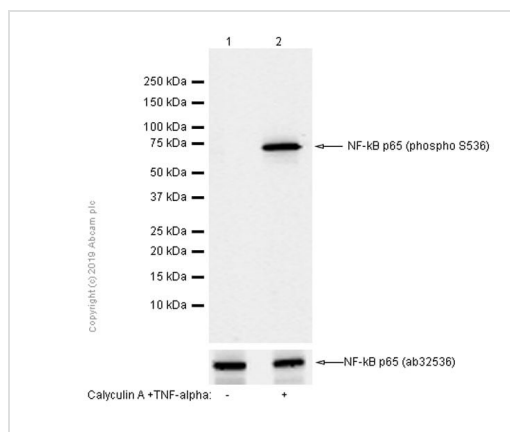
Lysates/proteins at 15 µg per lane.

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

Predicted band size: 65 kDa

Observed band size: 65 kDa



Western blot - Anti-NF-kB p65 (phospho S536) antibody [EP2294Y] (ab76302)

All lanes : Anti-NF-kB p65 (phospho S536) antibody [EP2294Y] (ab76302) at 1/1000 dilution

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

Lane 2 : HeLa (Human cervix adenocarcinoma epithelial cell) treated with Calyculin A and TNF-alpha whole cell lysate

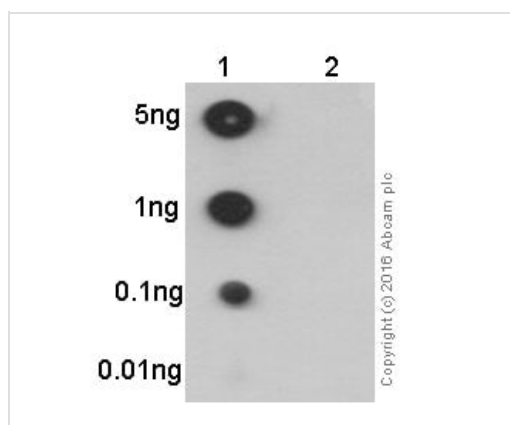
Lysates/proteins at 15 µg per lane.

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



Dot Blot - Anti-NF-kB p65 (phospho S536) antibody [EP2294Y] (ab76302)

Dot blot analysis of NF- kB p65 (phospho S536) phospho peptide (Lane 1) and NF- kB p65 non-phospho peptide (Lane 2) labeling NF-kB p65 (phospho S536) with ab76302 at a dilution of 1/1000. [ab97051](#)

(Peroxidase conjugated goat anti-rabbit IgG) (H+L) at 1/100 000 was used as the secondary antibody.

Blocking and diluting buffer: 5% NFD/MTBST.

Exposure time: 3 minutes.

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