abcam

Product datasheet

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

Recombinant RabMAb

7 Abreviews 160 References 6 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
Specificity	Stimulation may be required to allow detection of the phosphorylated protein. We recommend using NIH/3T3 (Mouse embryonic fibroblast) treated with 100nM Calyculin A for 30 minutes as a positive control.
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. This information is proprietary to Abcam and/or its suppliers.
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	Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

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

The Abpromise guarantee 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	★★★★☆ **(7)**	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 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.

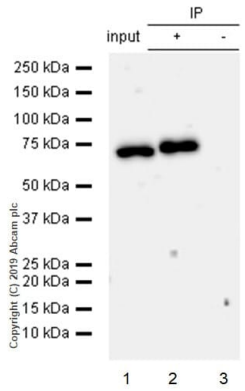
Post-translational modifications Ubiquitinated, leading to its proteasomal degradation. Degradation is required for termination of NF-kappa-B response.
Monomethylated at Lys-310 by SETD6. Monomethylation at Lys-310 is recognized by the ANK repeats of EHMT1 and promotes the formation of repressed chromatin 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



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)

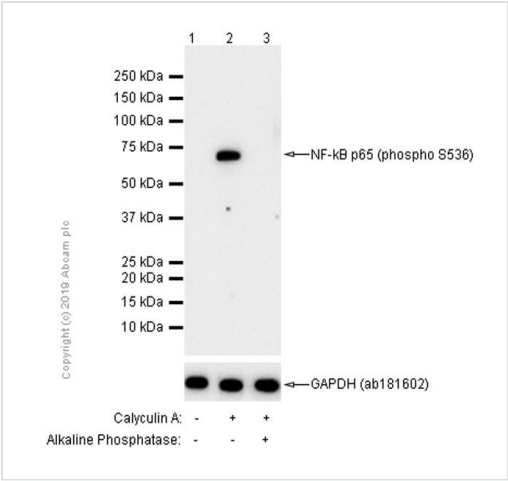
Lane 2 (+): ab76302 + Daudi whole cell lysate treated with calyculin A and tumor necrosis factor-alpha.

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)

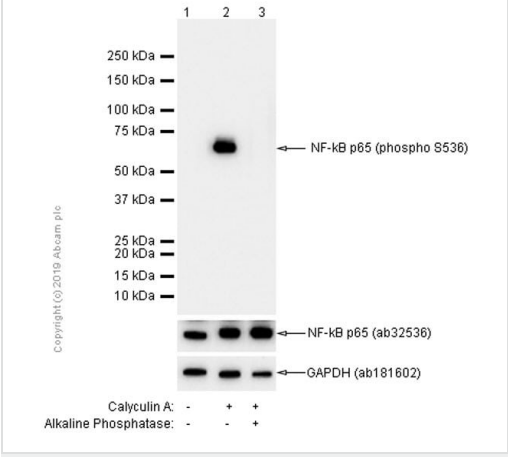
All lanes : Anti-NF-kB p65 (phospho S536) antibody [EP2294Y] (ab76302) at 1/1000 dilution

Lane 1 : C6 (Rat glial tumor glial cell) whole cell lysate
Lane 2 : C6 (Rat glial tumor glial cell) treated with 100nM Calyculin A for 30 minutes whole cell lysate
Lane 3 : C6 (Rat glial tumor glial 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



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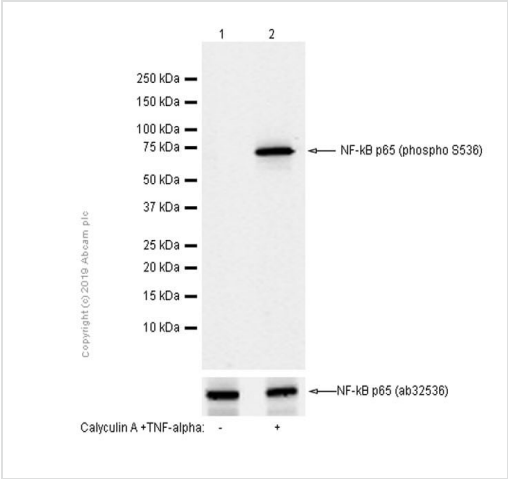

Western blot - Anti-NF-kB p65 (phospho S536) antibody [EP2294Y] (ab76302)

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

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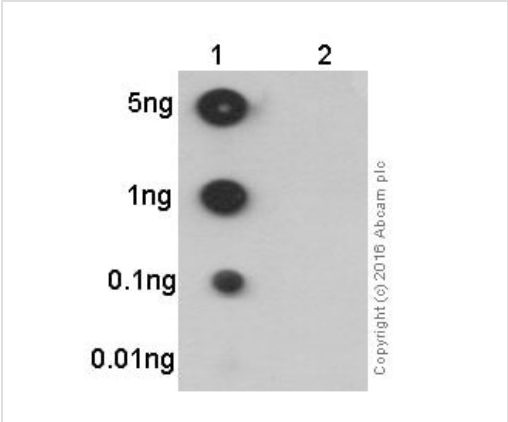
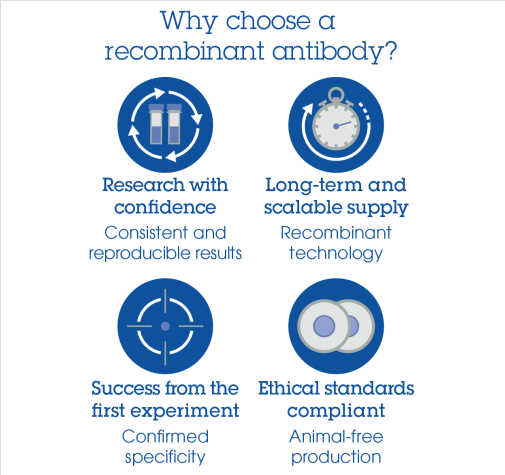


Dot Blot - Anti-NF-kB p65 (phospho S536) antibody [EP2294Y] (ab76302)

Dot blot analysis of INF- 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% NFDM/TBST.

Exposure time: 3 minutes.



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