**Product datasheet**

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

**Overview**

**Product name**  
Anti-NF-κB p65 (phospho S536) antibody [EP2294Y]

**Description**  
Rabbit monoclonal [EP2294Y] to NF-κB 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: Human

**Immunogen**  
Synthetic peptide (the amino acid sequence is considered to be commercially sensitive) within Human NF-κB p65 (phospho S536). The exact sequence is proprietary.  
(Peptide available as ab202905)

**Positive control**  
WB: HeLa and Daudi cell lysate treated with Calyculin A + TNF-alpha. IP: Daudi cell lysate treated with Calyculin A + TNF-alpha.

**General notes**  
A trial size is available to purchase for this antibody.  
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.

**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**  
P pH: 7.20  
Preservative: 0.01% Sodium azide
Constituents: 59% PBS, 40% Glycerol, 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.

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<td></td>
<td>1/1000.</td>
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<tr>
<td>IP</td>
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<td>1/20 - 1/30.</td>
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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 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.

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**Images**

All lanes: Anti-NF-kB p65 (phospho S536) antibody [EP2294Y] (ab76302) at 1/10000 dilution (purified)

Lane 1: HeLa whole cell lysate - untreated at 25 µg

Lane 2: HeLa whole cell lysate - treated with calyculin A and tumor necrosis factor-alpha at 10 µg

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 and dilution buffer: 5% NFDM/TBST.
Immunoprecipitation - Anti-NF-κB p65 (phospho S536) antibody [EP2294Y] (ab76302)

ab76302 (purified) at 1/30 immunoprecipitating NF-κB 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, ab131366 VeriBlot for IP (HRP) was used as the secondary antibody (1/1000).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM/TBST.

Dot blot analysis of NF-κB p65 (phospho S536) phospho peptide (Lane 1) and NF-κB p65 non-phospho peptide (Lane 2) labeling NF-κB 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.
Western blot - Anti-NF-kB p65 (phospho S536) antibody [EP2294Y] (ab76302)

All lanes: Anti-NF-kB p65 (phospho S536) antibody [EP2294Y] (ab76302) at 1/20000 dilution (unpurified)

Lane 1: Untreated Daudi cell lysate
Lane 2: Daudi cell lysate treated with Calyculin A + TNF-alpha

Lysates/proteins at 10 μg per lane.

Secondary
All lanes: HRP labelled goat anti-rabbit at 1/1000 dilution

Predicted band size: 65 kDa

Please note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

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