

Product datasheet

Anti-NF- κ B p65 antibody [E379] - Mouse IgG2 α (Chimeric) ab307840

KO VALIDATED Recombinant

4 Images

Overview

Product name	Anti-NF- κ B p65 antibody [E379] - Mouse IgG2 α (Chimeric)
Description	Mouse monoclonal [E379] to NF- κ B p65 - Chimeric
Host species	Mouse
Tested applications	Suitable for: IHC-P, WB Unsuitable for: ICC/IF or IP
Species reactivity	Reacts with: Human Does not react with: Mouse, Rat
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Wild-type HAP1 whole cell lysate. HeLa whole cell lysate. IHC-P: Human tonsil tissue. Human ovarian cancer tissue.
General notes	<p>ab307840 is a mouse monoclonal chimeric antibody.</p> <p>This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact orders@abcam.com.</p> <p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none">- High batch-to-batch consistency and reproducibility- Improved sensitivity and specificity- Long-term security of supply- Animal-free production <p>For more information see here.</p>

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 long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), 0.05% BSA, 59% PBS

Purity	Protein A purified
Clonality	Monoclonal
Clone number	E379
Isotype	IgG2a

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab307840 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
IHC-P		1/1000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB		1/1000. Predicted molecular weight: 60 kDa.

Application notes Is unsuitable for ICC/IF or IP.

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.

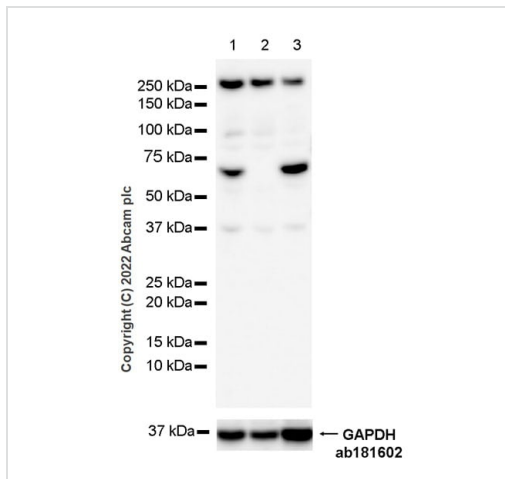
Phosphorylation at Ser-311 disrupts the interaction with EHMT1 and promotes transcription factor activity (By similarity). Phosphorylation on Ser-536 stimulates acetylation on Lys-310 and interaction with CBP; the phosphorylated and acetylated forms show enhanced transcriptional activity.

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



Western blot - Anti-NF-kB p65 antibody [E379] -
Mouse IgG2a (Chimeric) (ab307840)

All lanes : Anti-NF-kB p65 antibody [E379] - Mouse IgG2a
(Chimeric) (ab307840) at 1/1000 dilution

Lane 1 : Wild-type HAP1 (human chronic myelogenous leukemia
near-haploid cell line) cell lysate

Lane 2 : NF-kB p65 knockout HAP1 cell lysate

Lane 3 : HeLa (human epithelial cell line from cervical
adenocarcinoma) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Peroxidase-Conjugated Goat anti-Mouse IgG (H+L) at
1/10000 dilution

Predicted band size: 60 kDa

Observed band size: 65 kDa

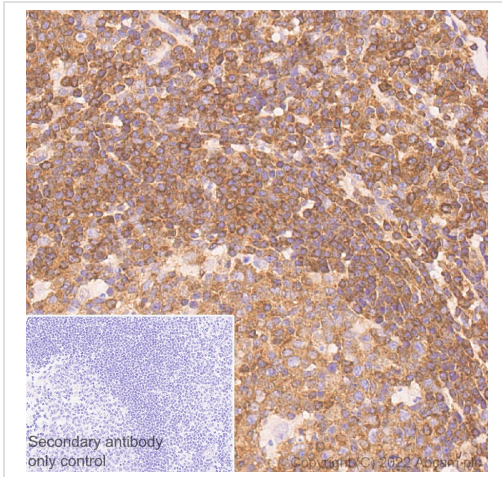
In Western blot, ab307840 was shown to bind specifically to NF-kB p65. A band was observed at 65 kDa in wild-type HAP1 cell lysates with whereas no signal observed at this size in NF-kB p65 knockout cell lysate.

The samples were run on a Bis-Tris gel.

In Western blot, anti-GAPDH antibody (**ab181602**) loading control staining at 1/200000 dilution.

Blocking/Dilution buffer: 5% NFD/MTBST.

Exposure time: 10 seconds.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NF-kB p65 antibody [E379] - Mouse IgG2a (Chimeric) (ab307840)

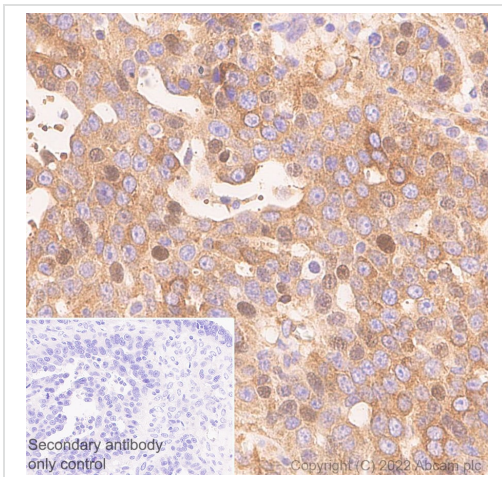
Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling NF-kB p65 with ab307840 at 1/1000 dilution (0.881 ug/mL) followed by ready to use LeicaDS9800 (Bond® Polymer Refine Detection).

Positive staining on human tonsil.

The section was incubated with ab307840 for 30 mins at room temperature, followed by anti-mouse IgG2a antibody for 8 mins during the LeicaDS9800 kit staining procedure. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is 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] - Mouse IgG2a (Chimeric) (ab307840)

Immunohistochemical analysis of paraffin-embedded human ovarian cancer tissue labeling NF-kB p65 with ab307840 at 1/1000 dilution (0.881 ug/mL) followed by ready to use LeicaDS9800 (Bond® Polymer Refine Detection).

Positive staining on human ovarian cancer.

The section was incubated with ab307840 for 30 mins at room temperature, followed by anti-mouse IgG2a antibody for 8 mins during the LeicaDS9800 kit staining procedure. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is 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.

Why choose a recombinant antibody?



Research with confidence
Consistent and reproducible results



Long-term and scalable supply
Recombinant technology



Success from the first experiment
Confirmed specificity



Ethical standards compliant
Animal-free production

Anti-NF-kB p65 antibody [E379] - Mouse IgG2a
(Chimeric) (ab307840)

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

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