abcam

Product datasheet

Anti-NFkB p100/NFKB2 (phospho S865) antibody ab31474

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Overview

Product name	Anti-NFkB p100/NFKB2 (phospho S865) antibody
Description	Rabbit polyclonal to NFkB p100/NFKB2 (phospho S865)
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, WB, IHC-P
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Rat
Immunogen	Synthetic peptide within Human NFkB p100/NFKB2 (phospho S865). The exact sequence is proprietary.
Positive control	WB: extracts of ovary cancer cells. IHC-P:breast carcinoma tissue.
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituents: PBS, 50% Glycerol (glycerin, glycerine), 0.87% Sodium chloride Without Mg2+ and Ca2+
Purity	Immunogen affinity purified
Purification notes	This antibody was affinity purified from rabbit antiserum by affinity chromatography using epitope specific phosphopeptide.

Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab31474 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

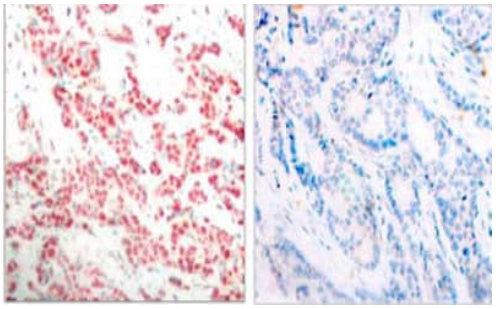
Application	Abreviews	Notes
ICC/IF		Use a concentration of 1 µg/ml.
WB		1/500 - 1/1000. Predicted molecular weight: 54 kDa.
IHC-P		1/50 - 1/100.

Target

Relevance NF-kappa-B is a pleiotropic transcription factor present in almost all cell types and is the endpoint of a series of signal transduction events that are initiated by a vast array of stimuli related to 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. 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. In a non-canonical activation pathway, the MAP3K14-activated CHUK/IKKA homodimer phosphorylates NFKB2/p100 associated with RelB, inducing its proteolytic processing to NFKB2/p52 and the formation of NF-kappa-B RelB-p52 complexes. The NF-kappa-B heterodimeric RelB-p52 complex is a transcriptional activator. The NF-kappa-B p52-p52 homodimer is a transcriptional repressor. NFKB2 appears to have dual functions such as cytoplasmic retention of attached NF-kappa-B proteins by p100 and generation of p52 by a cotranslational processing. The proteasome-mediated process ensures the production of both p52 and p100 and preserves their independent function. p52 binds to the kappa-B consensus sequence 5'-GGRNNYYCC-3', located in the enhancer region of genes involved in immune response and acute phase reactions. p52 and p100 are respectively the minor and major form; the processing of p100 being relatively poor. Isoform p49 is a subunit of the NF-kappa-B protein complex, which stimulates the HIV enhancer in synergy with p65. In concert with RELB, regulates the circadian clock by repressing the transcriptional activator activity of the CLOCK-ARNTL/BMAL1 heterodimer.

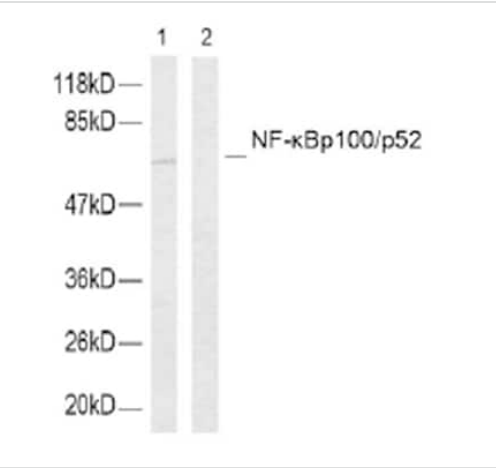
Cellular localization Cytoplasmic and Nuclear

Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NFkB p100/NFKB2 (phospho S865) antibody (ab31474)

Ab31474, at a dilution of 1/50, staining NFkB p100/NFKB2 in paraffin embedded human breast carcinoma tissue by Immunohistochemistry.
Left image: treated with ab31474.
Right image: treated with the same antibody preincubated with sythesized peptide.



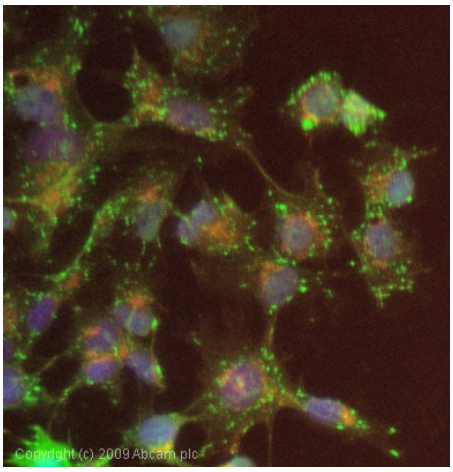
Western blot - Anti-NFkB p100/NFKB2 (phospho S865) antibody (ab31474)

All lanes : Anti-NFkB p100/NFKB2 (phospho S865) antibody (ab31474) at 1/500 dilution

Lane 1 : Extracts of ovary cancer cells (5-30ug).
Lane 2 : Extracts of ovary cancer cells (5-30ug). Antibody pre-incubated with synthesized peptide.

Secondary
All lanes : goat-anti-rabbit IgG-AP-conjugate.

Predicted band size: 54 kDa



Immunocytochemistry/ Immunofluorescence - Anti-NFkB p100/NFKB2 (phospho S865) antibody (ab31474)

ICC/IF image of ab31474 stained HepG2 cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab31474, 1µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

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