abcam

Product datasheet

Anti-NFkB p105 / p50 antibody [RP23040086] ab313430

Recombinant

4 Images

Overview

Product name	Anti-NFkB p105 / p50 antibody [RP23040086]
Description	Rabbit recombinant multiclonal [RP23040086] to NFkB p105 / p50
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, IHC-P, WB
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide within Human NFkB p105/ p50 aa 100-200. The exact immunogen sequence used to generate this antibody is proprietary information. If additional detail on the immunogen is needed to determine the suitability of the antibody for your needs, please **contact** our Scientific Support team to discuss your requirements. Database link: **P19838**
Positive control	ICC/IF: HeLa cells IHC: human breast carcinoma tissue WB: Hela cell lysate
General notes	Recombinant multiclonals are a mixture of recombinant antibodies co-expressed from a library of heavy and light chains. Recombinant multiclonal antibodies offer the sensitivity of polyclonal antibodies by recognising multiple epitopes, along with consistency of a recombinant antibody.

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	Preservative: 0.09% Sodium azide Constituent: 99.91% PBS
Purity	Protein A purified
Clonality	Recombinant Multiclonal
Clone number	RP23040086
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab313430 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 - 2 µg/ml.
IHC-P		1/20 - 1/200. Perform heat mediated antigen retrieval via the microwave method before commencing with IHC staining protocol.
WB		Use a concentration of 2 - 3 µg/ml. Predicted molecular weight: 105.356 kDa.

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 RelB-p50 complexes are transcriptional activators. The NF-kappa-B p50-p50 homodimer is a transcriptional repressor, but can act as a transcriptional activator when associated with BCL3. NFKB1 appears to have dual functions such as cytoplasmic retention of attached NF-kappa-B proteins by p105 and generation of p50 by a cotranslational processing. The proteasome-mediated process ensures the production of both p50 and p105 and preserves their independent function, although processing of NFKB1/p105 also appears to occur post-translationally. p50 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. In a complex with MAP3K8, NFKB1/p105 represses MAP3K8-induced MAPK signaling; active MAP3K8 is released by proteasome-dependent degradation of NFKB1/p105.

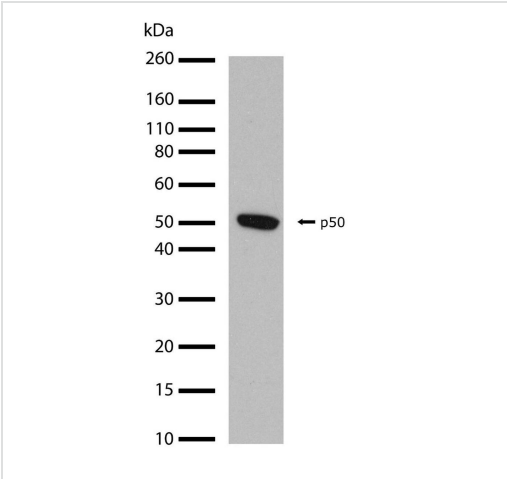
Sequence similarities Contains 7 ANK repeats.
Contains 1 death domain.
Contains 1 RHD (Rel-like) domain.

Domain The C-terminus of p105 might be involved in cytoplasmic retention, inhibition of DNA-binding, and transcription activation.
Glycine-rich region (GRR) appears to be a critical element in the generation of p50.

Post-translational While translation occurs, the particular unfolded structure after the GRR repeat promotes the

modifications	generation of p50 making it an acceptable substrate for the proteasome. This process is known as cotranslational processing. The processed form is active and the unprocessed form acts as an inhibitor (I kappa B-like), being able to form cytosolic complexes with NF-kappa B, trapping it in the cytoplasm. Complete folding of the region downstream of the GRR repeat precludes processing. Phosphorylation at 'Ser-903' and 'Ser-907' primes p105 for proteolytic processing in response to TNF-alpha stimulation. Phosphorylation at 'Ser-927' and 'Ser-932' are required for BTRC/BTRCP-mediated proteolysis. Polyubiquitination seems to allow p105 processing. S-nitrosylation of Cys-61 affects DNA binding.
Cellular localization	Nucleus. Cytoplasm. Nuclear, but also found in the cytoplasm in an inactive form complexed to an inhibitor.

Images



Western blot - Anti-NFkB p105 / p50 antibody [RP23040086] (ab313430)

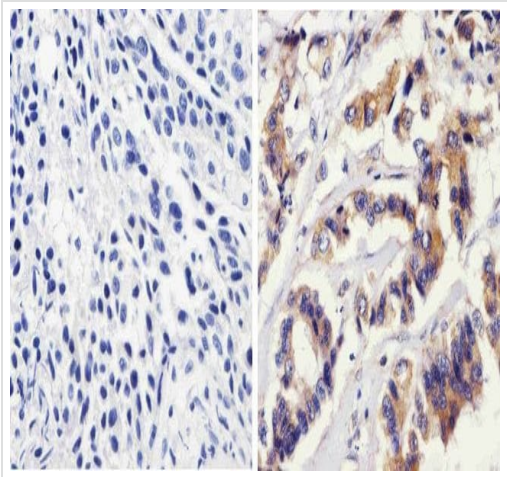
Anti-NFkB p105 / p50 antibody [RP23040086] (ab313430) at 2 µg/ml + HeLa whole cell lysate

Secondary

HRP-conjugated Goat anti-Rabbit secondary antibody

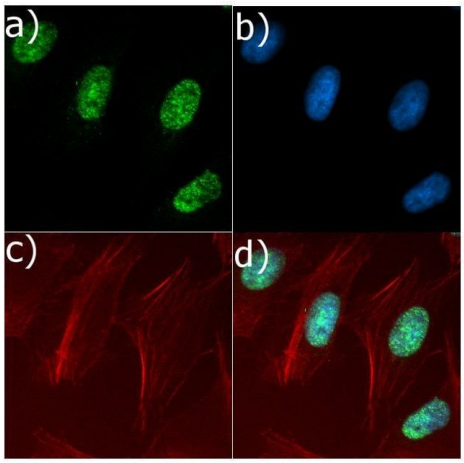
Predicted band size: 105.356 kDa

Observed band size: 50 kDa



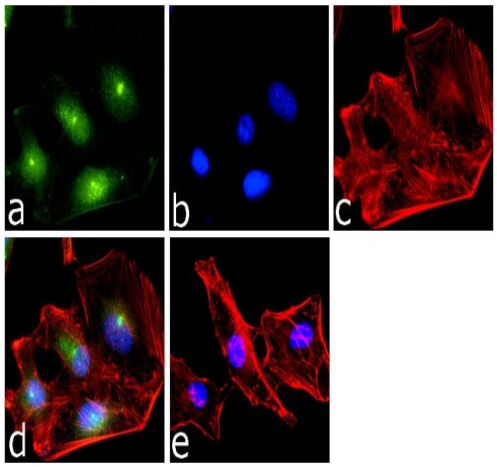
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NFkB p105 / p50 antibody [RP23040086] (ab313430)

Immunohistochemical analysis of paraffin-embedded human breast carcinoma (right) labeling NFkB p50 with ab313430 at 1/100 dilution compared to a negative control without primary antibody (left). To expose target proteins, antigen retrieval was performed using 10 mM sodium citrate (pH 6.0), microwaved for 8-15 min. Following antigen retrieval, tissues were blocked in 3% H2O2-methanol for 15 min at room temperature, washed with ddH2O and PBS, and then probed with ab313430. Counterstained with hematoxylin and dehydrated with ethanol and xylene to prep for mounting.



Immunocytochemistry/ Immunofluorescence - Anti-NFkB p105 / p50 antibody [RP23040086] (ab313430)

Immunofluorescent analysis of HeLa cells fixed using 4% formaldehyde (reconstituted in 1X PBS) for 10 min at room temperature and permeabilized using 0.1 % Triton X-100 in PBS for 15 min at room temperature labeling NFkB p50 with ab313430 followed by detection using an Alexa Fluor 488-conjugated Goat anti-Rabbit secondary antibody (green) (Image A). Nuclei were stained using DAPI (Image B) and actin stained with Alexa Fluor 594 phalloidin (red) (image C). Image D is a composite image showing nuclear localization of p50.



Immunocytochemistry/ Immunofluorescence - Anti-NFkB p105 / p50 antibody [RP23040086] (ab313430)

Immunofluorescent analysis of 70% confluent log phase HeLa cells fixed with 4% paraformaldehyde for 15 minutes, permeabilized with 0.25% Triton X-100 for 10 minutes, and blocked with 5% BSA for 1 hour at room temperature, labeling NFkB p50 with ab313430 at 1 µg/ml followed by Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 488 conjugate at a dilution of 1/2000 for 45 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with DAPI. F-actin (Panel c: red) was stained with Alexa Fluor 555 Rhodamine Phalloidin at 1/300. Panel d is a merged image showing Nuclear localization. Panel e is a no primary antibody control. The images were captured at 60X magnification.

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