abcam

Product datasheet

Anti-NFkB p105 / p50 antibody [EPR25226-156] ab283688

KO VALIDATED Recombinant RabMAb

2 References 8 Images

Overview

Product name	Anti-NFkB p105 / p50 antibody [EPR25226-156]
Description	Rabbit monoclonal [EPR25226-156] to NFkB p105 / p50
Host species	Rabbit
Tested applications	**Suitable for:** WB, IP, IHC-P **Unsuitable for:** Flow Cyt (Intra) or ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Wild-type HAP1 and HeLa whole cell lysate; Human heart tissue lysate. IHC-P: Human prostate hyperplasia, Human tonsil and Human colon cancer. IP: HAP1 cells.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. 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: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number EPR25226-156

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab283688 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
WB		1/1000. Predicted molecular weight: 105 kDa.
IP		1/30.
IHC-P		1/4000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Application notes Is unsuitable for Flow Cyt (Intra) or ICC/IF.

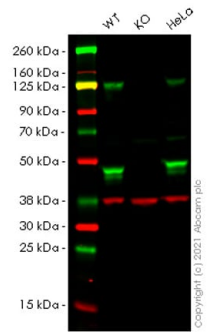
Target

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 modifications	While translation occurs, the particular unfolded structure after the GRR repeat promotes the 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 [EPR25226-156] (ab283688)

All lanes : Anti-NFkB p105 / p50 antibody [EPR25226-156] (ab283688) at 1/1000 dilution

Lane 1 : Wild-type HAP1 (human chronic myelogenous leukemia near-haploid cell) whole cell lysate
Lane 2 : NFkB p105 / p50 knockout HAP1 whole cell lysate
Lane 3 : HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (IRDye® 800CW) (**ab216773**) and Goat Anti-Mouse IgG H&L (IRDye® 680RD) (**ab216776**) at 1/10000 dilution

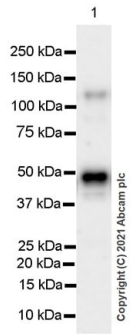
Predicted band size: 105 kDa
Additional bands at: 120 kDa (possible immature (unprocessed)), 50 kDa (possible mature (processed) protein)

Blocking and diluting buffer and concentration: Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBSLanes 1 - 3: Merged signal (red and green). Green - ab283688 observed at 120, 50 kDa. Red - loading control, **ab8245**, was observed at 36

kDa.

ab283688 was shown to specifically react with NFkB p105 / p50 when NFkB p105 / p50 knockout samples were used. Wild-type and NFkB p105 / p50 knockout samples were subjected to SDS-PAGE.

ab283688 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated at 4°C overnight at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-NFkB p105 / p50 antibody [EPR25226-156] (ab283688)

Anti-NFkB p105 / p50 antibody [EPR25226-156] (ab283688) at 1/1000 dilution + Human heart tissue lysate at 20 µg

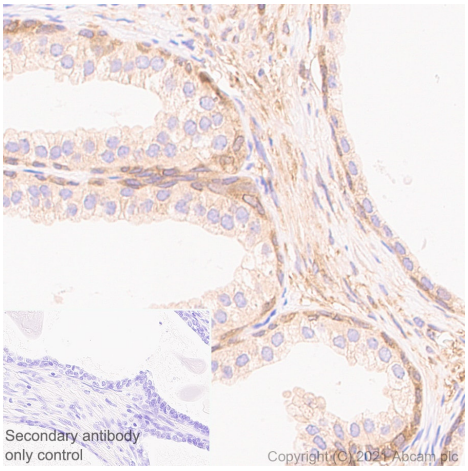
Secondary

VeriBlot for IP secondary antibody(HRP)(**ab131366**) at 1/1000 dilution

Predicted band size: 105 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST

Exposure time: 59 seconds

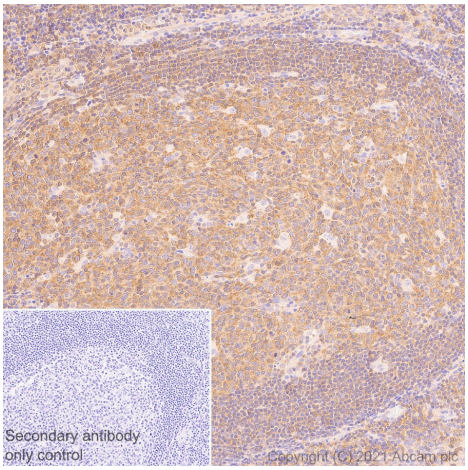


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NFkB p105 / p50 antibody [EPR25226-156] (ab283688)

Immunohistochemical analysis of paraffin-embedded Human prostate hyperplasia tissue labelling NFkB p105/p50 with ab283688 at 1/4000 (0.134 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Cytoplasmic staining on human prostate hyperplasia. The section was incubated with ab283688 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

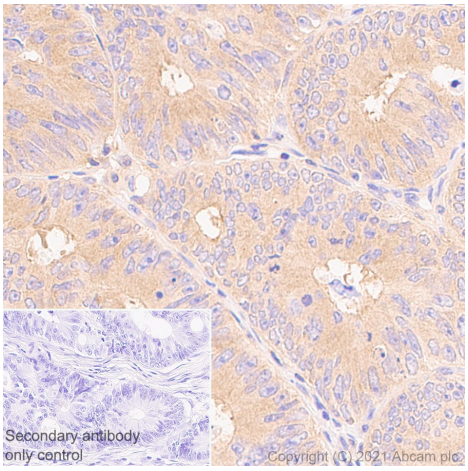


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NFkB p105 / p50 antibody [EPR25226-156] (ab283688)

Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labelling NFkB p105/p50 with ab283688 at 1/4000 (0.134 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on human tonsil. The section was incubated with ab283688 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

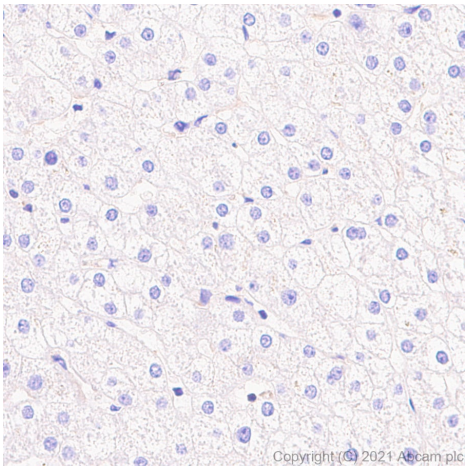


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NFkB p105 / p50 antibody [EPR25226-156] (ab283688)

Immunohistochemical analysis of paraffin-embedded Human colon cancer tissue labelling NFkB p105/p50 with ab283688 at 1/4000 (0.134 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) was used. Cytoplasmic staining on human colon cancer. The section was incubated with ab283688 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

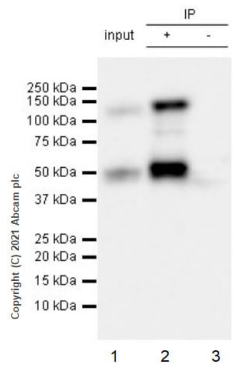


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NFkB p105 / p50 antibody [EPR25226-156] (ab283688)

Immunohistochemical analysis of paraffin-embedded Human liver tissue labelling NFkB p105/p50 with ab283688 at 1/4000 (0.134 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) was used. Negative control: No staining on human liver. The section was incubated with ab283688 for 30 mins at room temperature.The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Immunoprecipitation - Anti-NFkB p105 / p50 antibody [EPR25226-156] (ab283688)

NFκB p105/p50 was immunoprecipitated from 0.35 mg HAP1 (human chronic myelogenous leukemia near-haploid cell) whole cell lysate 10 µg with ab283688 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab283688 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(**ab131366**) was used at 1/5000 dilution.

Lane 1: HAP1 (human chronic myelogenous leukemia near-haploid cell) whole cell lysate 10 µg

Lane 2: ab283688 IP in HAP1 whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab283688 in HAP1 whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 7.75 seconds

Anti-NFkB p105 / p50 antibody [EPR25226-156] (ab283688)

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