abcam

Product datasheet

Anti-NFkB p100/NFKB2 antibody [EPR4686] ab109440

KO VALIDATED Recombinant RabMAb

1 Abreviews 5 References 6 Images

Overview

Product name	Anti-NFkB p100/NFKB2 antibody [EPR4686]
Description	Rabbit monoclonal [EPR4686] to NFkB p100/NFKB2
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, WB **Unsuitable for:** Flow Cyt or IHC-P
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse
Immunogen	Synthetic peptide within Human NFkB p100/NFKB2 aa 700 to the C-terminus. The exact sequence is proprietary. Database link: **Q00653**
Positive control	WB: Jurkat, HeLa, ECV-304, HepG2, HCT116 and MCF7 cell lysates ICC/IF: Wild-type 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**. Rat: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.05% Sodium azide Constituents: 0.1% BSA, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue

	culture supernatant
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR4686
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab109440 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		1/250.
WB	★★★★☆ (1)	1/10000 - 1/50000. Detects a band of approximately 110 kDa (predicted molecular weight: 97 kDa).

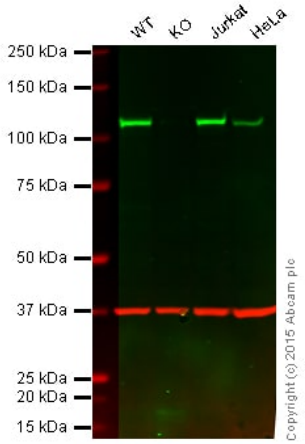
Application notes Is unsuitable for Flow Cyt or IHC-P.

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



Western blot - Anti-NFkB p100/NFKB2 antibody [EPR4686] (ab109440)

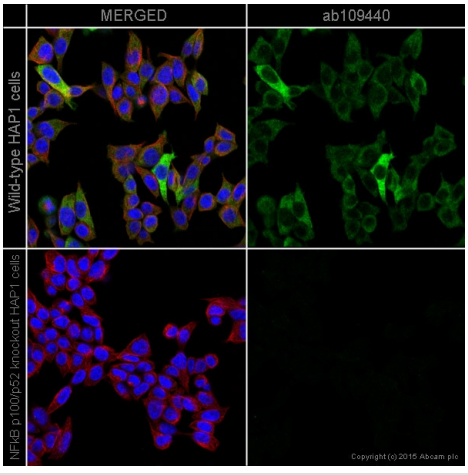
Lane 1: Wild-type HAP1 cell lysate (20 µg)

Lane 2: NFκB p100 knockout HAP1 cell lysate (20 µg)

Lane 3: Jurkat cell lysate (20 µg)

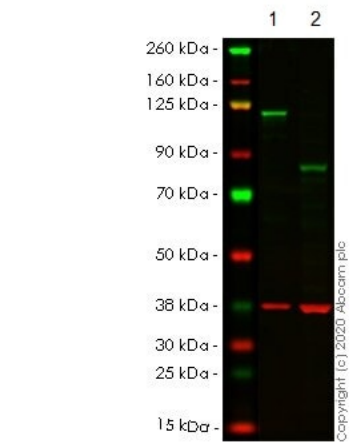
Lane 4: HeLa cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab109440 observed at 100 kDa. Red - loading control, **ab8245**, observed at 37 kDa.
ab109440 was shown to specifically react with NFκB p100 when NFκB p100 knockout samples were used. Wild-type and NFκB p100 knockout samples were subjected to SDS-PAGE. ab109440 and ab109440 (loading control to GAPDH) were diluted 1/10000 and 1/2000 respectively and incubated overnight at 4°C. 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 1/10000 dilution for 1 h at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-NFkB p100/NFKB2 antibody [EPR4686] (ab109440)

ab109440 staining NFkB p100/p52 in wild-type HAP1 cells (top panel) and NFkB p100/p52 knockout HAP1 cells (bottom panel). The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab109440 at 1/250 dilution and **ab195889** at 1/250 dilution (shown in pseudo colour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.



Western blot - Anti-NFkB p100/NFKB2 antibody [EPR4686] (ab109440)

All lanes : Anti-NFkB p100/NFKB2 antibody [EPR4686] (ab109440) at 1/1000 dilution

Lane 1 : Wild-type HCT116 cell lysate
Lane 2 : NFKB2 CRISPR/Cas9 edited HCT116 cell lysate

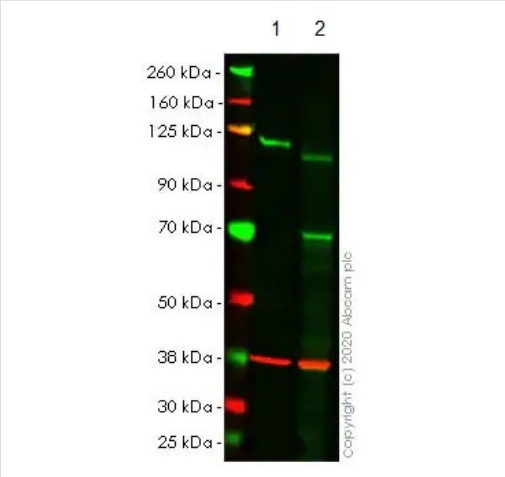
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 97 kDa
Observed band size: 120 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab109440 observed at 120 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab109440 was shown to react with NFkB p100/NFKB2 in wild-type HCT116 cells in western blot. The band observed in CRISPR/Cas9 edited cell line **ab266883** (CRISPR/Cas9 edited cell lysate **ab257245**) lane below 97kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type HCT116 and NFKB2 CRISPR/Cas9 edited HCT116 cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab109440 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at a 1 in 1000 dilution and a 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 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-NFkB p100/NFKB2 antibody [EPR4686] (ab109440)

All lanes : Anti-NFkB p100/NFKB2 antibody [EPR4686] (ab109440) at 1/1000 dilution

Lane 1 : Wild-type HepG2 cell lysate
Lane 2 : NFKB2 CRISPR/Cas9 edited HepG2 cell lysate

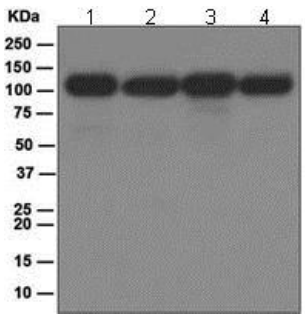
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 97 kDa
Observed band size: 120 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab109440 observed at 120 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab109440 was shown to react with NFkB p100/NFKB2 in wild-type HepG2 cells in western blot. The band observed in CRISPR/Cas9 edited cell line **ab262323** (CRISPR/Cas9 edited cell lysate **ab257247**) lane below 97kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type HepG2 and NFKB2 CRISPR/Cas9 edited HepG2 cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab109440 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at a 1 in 1000 dilution and a 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 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-NFkB p100/NFKB2 antibody [EPR4686] (ab109440)

All lanes : Anti-NFkB p100/NFKB2 antibody [EPR4686] (ab109440) at 1/10000 dilution

Lane 1 : Jurkat cell lysate
Lane 2 : HeLa cell lysate
Lane 3 : ECV-304 cell lysate
Lane 4 : MCF7 cell lysate

Lysates/proteins at 10 µg per lane.

Predicted band size: 97 kDa
Observed band size: 110 kDa

Anti-NFkB p100/NFKB2 antibody [EPR4686] (ab109440)

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours

- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors