abcam

Product datasheet

Anti-NFkB p100/NFKB2 antibody [EPR4686] - BSA and Azide free ab174482

KO 評価済 リコンビナント RabMAb

1 References 画像数 6

製品の概要

製品名	Anti-NFkB p100/NFKB2 antibody [EPR4686] - BSA and Azide free
製品の詳細	Rabbit monoclonal [EPR4686] to NFkB p100/NFKB2 - BSA and Azide free
由来種	Rabbit
アプリケーション	**適用あり:** WB, ICC/IF **適用なし:** Flow Cyt or IHC-P
種交差性	**交差種:** Human **交差が予測される動物種:** Mouse
免疫原	Synthetic peptide within Human NFkB p100/NFKB2 aa 700 to the C-terminus. The exact sequence is proprietary. Database link: **Q00653**
ポジティブ・コントロール	WB: Jurkat, HeLa, ECV-304, HepG2, HCT116 and MCF7 cell lysates ICC/IF: Wild-type HAP1 cells.
特記事項	ab174482 is the carrier-free version of **ab109440**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C. Do Not Freeze.
バッファー	Constituent: PBS
キャリア・フリー	はい
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR4686
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab174482の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		Use at an assay dependent concentration. Detects a band of approximately 110 kDa (predicted molecular weight: 97 kDa).
ICC/IF		Use at an assay dependent concentration.

追加情報 Is unsuitable for Flow Cyt or IHC-P.

ターゲット情報

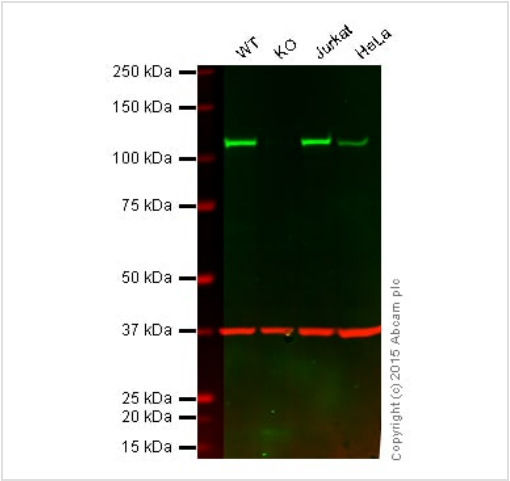
関連性 NF-kappa-B is a pleiotropic transcription factor present in almost all cell types and is the endpoint of a series of signal transduction events that are initiated by a vast array of stimuli related to many biological processes such as inflammation, immunity, differentiation, cell growth, tumorigenesis 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.

細胞内局在

Cytoplasmic and Nuclear

画像



Western blot - Anti-NFkB p100/NFKB2 antibody [EPR4686] - BSA and Azide free (ab174482)

This data was developed using **ab109440**, the same antibody clone in a different buffer formulation.

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

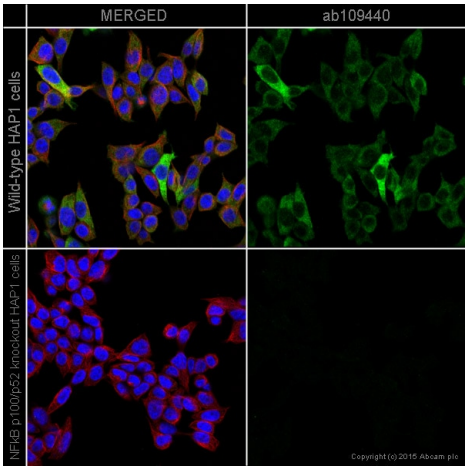
Lane 2 NF?B p100 knockout HAP1 cell lysate (20 µg)

Lane 3 Jurkat cell lysate (20 µg)

Lane 4 HeLa cell lysate (20 µg)

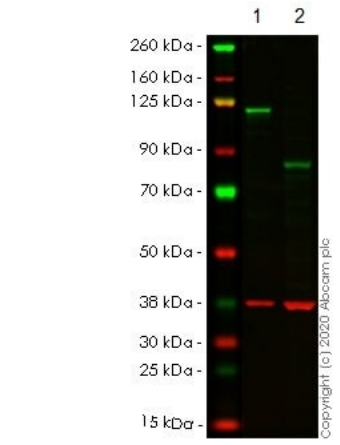
Lanes 1 - 4 Merged signal (red and green). Green - **ab109440** observed at 100 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab109440 was shown to specifically react with NF?B p100 when NF?B p100 knockout samples were used. Wild-type and NFB 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] - BSA and Azide free (ab174482)

This data was developed using **ab109440**, the same antibody clone in a different buffer formulation.**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] - BSA and Azide free (ab174482)

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.

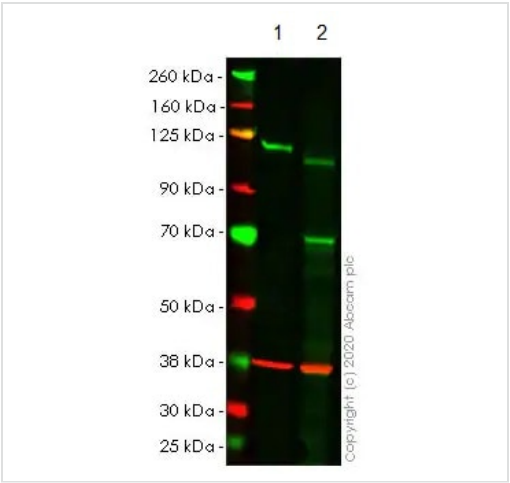
Predicted band size: 97 kDa
Observed band size: 120 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab109440**).

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] - BSA and Azide free (ab174482)

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.

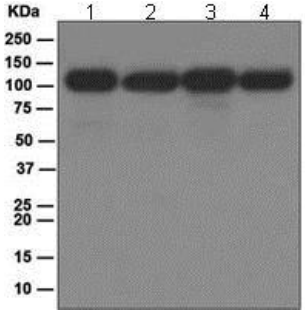
Predicted band size: 97 kDa
Observed band size: 120 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109440**).

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] - BSA and Azide free (ab174482)

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

This data was developed using **ab109440**, the same antibody clone in a different buffer formulation.

Anti-NFkB p100/NFKB2 antibody [EPR4686] - BSA and Azide free (ab174482)

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