abcam

Product datasheet

Anti-NFkB p100/NFKB2 antibody [EPR4686] ab109440



★★★★★ 1 Abreviews 5 References 画像数 6

製品の概要

免疫原

製品名 Anti-NFkB p100/NFKB2 antibody [EPR4686]

製品の詳細 Rabbit monoclonal [EPR4686] to NFkB p100/NFKB2

由来種 Rabbit

アプリケーション 適用あり: ICC/IF, WB

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 4

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 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

保存方法 Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.

バッファー pH: 7.20

Preservative: 0.05% Sodium azide

Constituents: 0.1% BSA, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue

culture supernatant

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 <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab109440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
ICC/IF		1/250.
WB	★★★★ (1)	1/10000 - 1/50000. Detects a band of approximately 110 kDa (predicted molecular weight: 97 kDa).

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 domaincontaining 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, Likappa-B is phosphorylated by Likappa-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

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

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

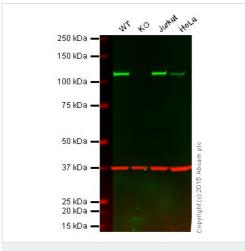
Lane 3: Jurkat cell lysate (20 µg)

Lane 4: HeLa cell lysate (20 µg)

細胞内局在

Cytoplasmic and Nuclear

画像



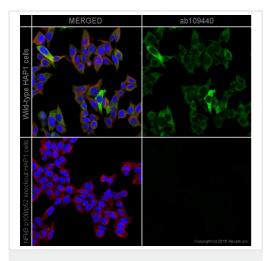
Western blot - Anti-NFkB p100/NFKB2 antibody [EPR4686] (ab109440)



observed at 100 kDa. Red - loading control, ab8245, observed at 37 kDa. ab109440 was shown to specifically react with NFkB p100

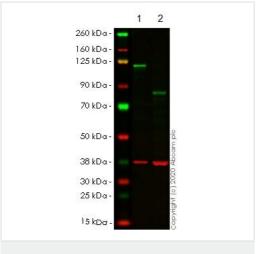
Lanes 1 - 4: Merged signal (red and green). Green - ab109440

when NFkB p100 knockout samples were used. Wild-type and NFkB 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

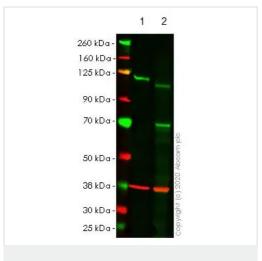
Lysates/proteins at 20 µg per lane.

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 lgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG 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

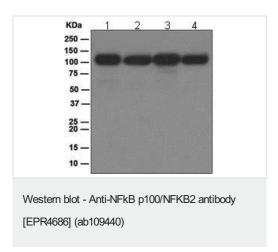
Lysates/proteins at 20 µg per lane.

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 lgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



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



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