abcam

Product datasheet

Human NFKB2 (NFkB p100/NFKB2) knockout Hep G2 cell lysate ab257247

画像数 3

製品の概要

製品名	Human NFKB2 (NFkB p100/NFKB2) knockout Hep G2 cell lysate
製品の概要	Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images.
Parental Cell Line	HepG2
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 28 bp deletion in exon12.
Passage number	<20
Knockout validation	Sanger Sequencing
Reconstitution notes	To use as WB control, resuspend the lyophilizate in 50 µL of LDS* Sample Buffer to have a final concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M DTT. **Usage of SDS sample buffer is not recommended with these lyophilized lysates.*
特記事項	**Lysate preparation:** Our lysates are made using RIPA buffer to which we add a protease inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found **here**. Please refer to our lysis protocol for further details on how our lysates are prepared. **User storage instructions:** Lyophilizate may be stored at 4°C. After reconstitution, store at -20°C for short-term storage or -80°C for long-term storage. Access thousands of knockout cell lysates, generated from commonly used cancer cell lines. **See here for more information on knockout cell lysates.** Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses. This product is subject to limited use licenses from The Broad Institute and ERS Genomics Limited, and is developed with patented technology. For full details of the limited use licenses and relevant patents please refer to our **limited use license** and **patent pages**.
アプリケーション	**適用あり:** WB

製品の特性

保存方法 Store at -80°C. Please refer to protocols.

内容	1 kit
ab263508 - Human NFKB2 knockout HepG2 cell lysate	1 x 100µg
ab263911 - Human wild-type HepG2 cell lysate	1 x 100µg

Cell type epithelial

Disease Hepatocellular Carcinoma

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

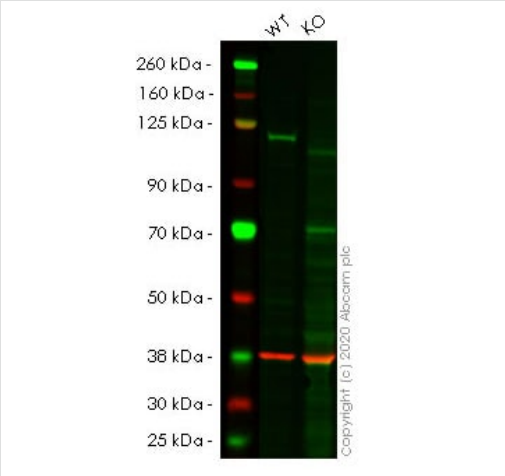
アプリケーション

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ab257247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. Predicted molecular weight: 97 kDa. Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images.

画像



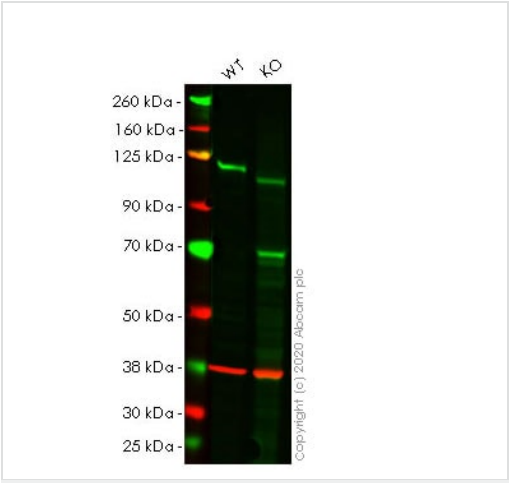
Western blot - Human NFKB2 (NFkB p100/NFKB2) knockout HEPG2 cell lysate (ab257247)

Lane 1: Wild-type HepG2 cell lysate (20µg)

Lane 2: NFKB2 knockout HepG2 cell lysate (20µg)

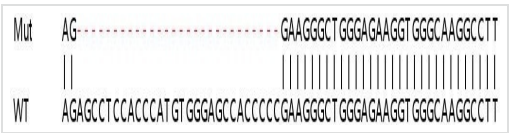
Lanes 1- 2: Merged signal (red and green). Green - **ab175192** observed at 120 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab175192 Anti-NFkB p100/NFKB2 antibody [EPR4686-66] was shown to specifically react with NFkB p100/NFKB2 in wild-type HepG2 cells in western blot. The band observed in the knockout cell line **ab262323** (knockout cell lysate ab257247) lane below 97kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type and NFkB p100/NFKB2 knockout samples were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab175192** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 10000 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 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human NFKB2 (NFkB p100/NFKB2) knockout HEPG2 cell lysate (ab257247)

Lane 1: Wild-type HepG2 cell lysate (20µg)
Lane 2: NFKB2 knockout HepG2 cell lysate (20µg)
Lanes 1- 2: Merged signal (red and green). Green - **ab109440** observed at 120 kDa. Red - loading control, **ab8245** observed at 37 kDa.
ab109440 Anti-NFkB p100/NFKB2 antibody [EPR4686] was shown to specifically react with NFkB p100/NFKB2 in wild-type HepG2 cells in western blot. The band observed in the knockout cell line **ab262323** (knockout cell lysate ab257247) lane below 97kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type and NFkB p100/NFKB2 knockout samples 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 1 in 1000 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 20000 dilution for 1 hour at room temperature before imaging.



Sanger Sequencing - Human NFKB2 knockout HEPG2 cell lysate (ab257247)

Homozygous: 28 bp deletion in exon12

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