


## Product datasheet

### Anti-NFkB p100/NFKB2 antibody [EPR4686-66] ab175192

KO **VALIDATED** Recombinant RabMAb

★★★★★ [1 Abreviews](#) [7 Images](#)

#### Overview

<b>Product name</b>	Anti-NFkB p100/NFKB2 antibody [EPR4686-66]
<b>Description</b>	Rabbit monoclonal [EPR4686-66] to NFkB p100/NFKB2
<b>Host species</b>	Rabbit
<b>Tested applications</b>	<b>Suitable for:</b> ICC/IF, IP, WB <b>Unsuitable for:</b> Flow Cyt or IHC-P
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Human <b>Predicted to work with:</b> Rat 
<b>Immunogen</b>	Recombinant fragment within Human NFkB p100/NFKB2. The exact sequence is proprietary. Database link: <a href="#">Q00653</a>
<b>Positive control</b>	WB: Jurkat, HeLa, Daudi, HCT116 and MCF7 cell lysates. ICC/IF: Wild-type HAP1 cells. IP: Jurkat Cells
<b>General notes</b>	This product is a recombinant monoclonal antibody, which offers several advantages including: <ul style="list-style-type: none"> <li>- High batch-to-batch consistency and reproducibility</li> <li>- Improved sensitivity and specificity</li> <li>- Long-term security of supply</li> <li>- Animal-free production</li> </ul> For more information <a href="#">see here</a> . Our RabMAb <sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a> .

#### Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
<b>Storage buffer</b>	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 9% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA, 50% Tissue culture supernatant
<b>Purity</b>	Protein A purified

<b>Clonality</b>	Monoclonal
<b>Clone number</b>	EPR4686-66
<b>Isotype</b>	IgG

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab175192 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.
IP		1/10 - 1/100.
WB	★★★★★ (1)	1/10000 - 1/50000. Predicted molecular weight: 97 kDa.

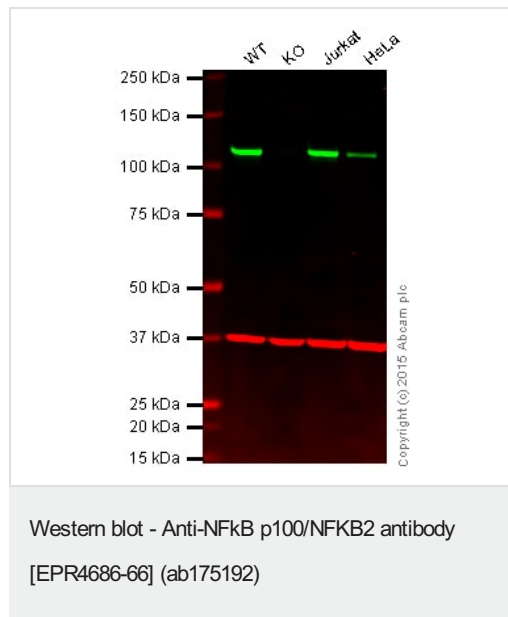
**Application notes** Is unsuitable for Flow Cyt or IHC-P.

## 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.

## Images



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

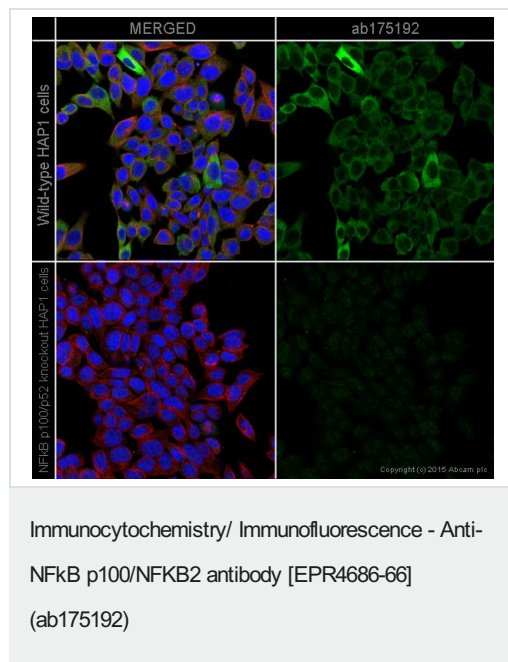
**Lane 2:** NFκB p100 knockout HAP1 cell lysate (20 µg)

**Lane 3:** Jurkat cell lysate (20 µg)

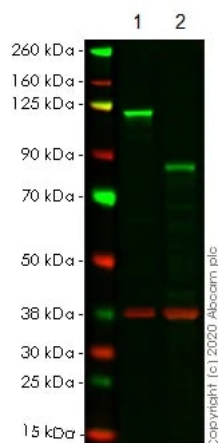
**Lane 4:** HeLa cell lysate (20 µg)

**Lanes 1 - 4:** Merged signal (red and green). Green - ab175192 observed at 97 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab175192 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. ab175192 and **ab8245** (loading control to GAPDH) were diluted 1/10 000 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.



ab175192 staining NFκB p100/NFKB2 in wild-type HAP1 cells (top panel) and NFκB p100/NFKB2 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 ab175192 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 [EPR4686-66] (ab175192)

**All lanes :** Anti-NFkB p100/NFKB2 antibody [EPR4686-66] (ab175192) 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.

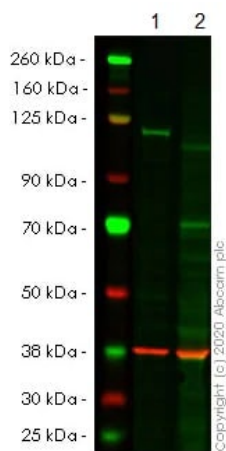
Performed under reducing conditions.

**Predicted band size:** 97 kDa

**Observed band size:** 120 kDa

**Lanes 1- 2:** Merged signal (red and green). Green - ab175192 observed at 97 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) observed at 37 kDa.

ab175192 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 HCT117 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. ab175192 and Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) were incubated overnight at 4°C at a 1 in 10000 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 [EPR4686-66] (ab175192)

**All lanes :** Anti-NFkB p100/NFKB2 antibody [EPR4686-66] (ab175192) 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.

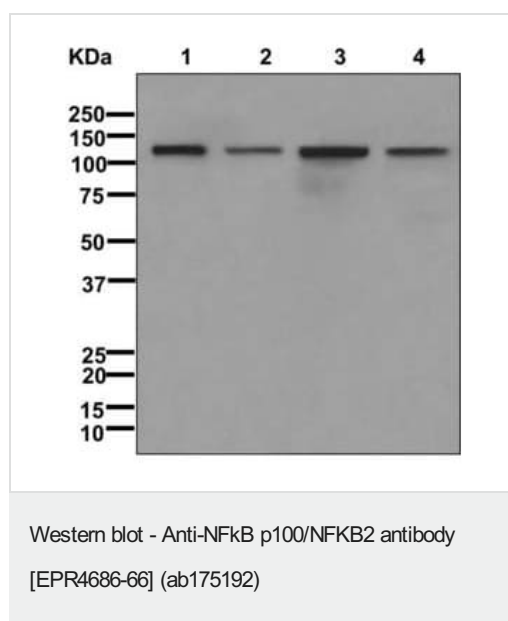
Performed under reducing conditions.

**Predicted band size:** 97 kDa

**Observed band size:** 120 kDa

**Lanes 1- 2:** Merged signal (red and green). Green - ab175192 observed at 120 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) observed at 37 kDa.

ab175192 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. ab175192 and Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) were incubated overnight at 4°C at a 1 in 10000 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.



**All lanes :** Anti-NFkB p100/NFKB2 antibody [EPR4686-66] (ab175192) at 1/10000 dilution

**Lane 1 :** Jurkat cell lysate

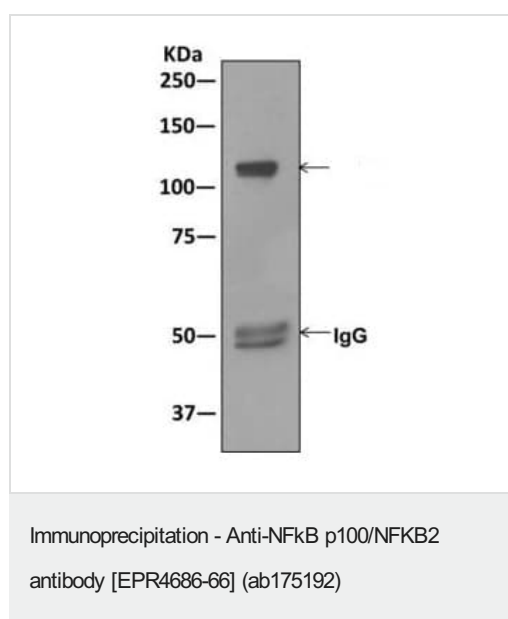
**Lane 2 :** HeLa cell lysate

**Lane 3 :** Daudi cell lysates

**Lane 4 :** MCF7 cell lysate

Lysates/proteins at 10 µg per lane.

**Predicted band size:** 97 kDa



Western blot analysis on immunoprecipitation pellet from Jurkat cell lysate labeling NFkB p100/NFKB2 with ab175192 at 1/10 dilution.

### Why choose a recombinant antibody?



**Research with confidence**  
Consistent and reproducible results



**Long-term and scalable supply**  
Recombinant technology



**Success from the first experiment**  
Confirmed specificity



**Ethical standards compliant**  
Animal-free production

Anti-NFkB p100/NFkB2 antibody [EPR4686-66]  
(ab175192)

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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