

## Product datasheet

# Anti-NFkB p100/NFKB2 antibody [EPR18756] ab191594

Recombinant RabMAb

[2 References](#) [9 Images](#)

### Overview

<b>Product name</b>	Anti-NFkB p100/NFKB2 antibody [EPR18756]
<b>Description</b>	Rabbit monoclonal [EPR18756] to NFkB p100/NFKB2
<b>Host species</b>	Rabbit
<b>Tested applications</b>	<b>Suitable for:</b> Flow Cyt (Intra), ICC/IF, IP, WB
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Rat, Human
<b>Immunogen</b>	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
<b>Positive control</b>	WB: Human liver and kidney lysates; HeLa, Jurkat, MCF7, Daudi, C6 and NIH/3T3 whole cell lysates; Mouse heart and kidney lysates; Rat brain, heart, kidney and spleen lysates. ICC/IF: HeLa and NIH/3T3 cell lines. Flow Cyt (intra): HeLa cell line. IP: HeLa whole cell lysate.
<b>General notes</b>	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <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> <p>For more information <a href="#">see here</a>.</p> <p>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>.</p>

### 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: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
<b>Purity</b>	Protein A purified
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	EPR18756

Isotype

IgG

## Applications

### The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab191594 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
Flow Cyt (Intra)		1/60.
ICC/IF		1/250.
IP		1/20.
WB		1/1000. Detects a band of approximately 110 kDa (predicted molecular weight: 97 kDa).

## Target

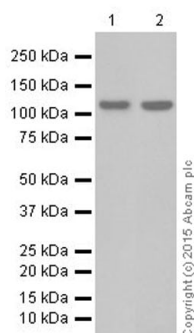
### Relevance

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'-GGRNYYCC-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 [EPR18756] (ab191594)

**All lanes** : Anti-NFkB p100/NFKB2 antibody [EPR18756] (ab191594) at 1/1000 dilution

**Lane 1** : Human liver lysate

**Lane 2** : Human kidney lysate

Lysates/proteins at 10 µg per lane.

### Secondary

**All lanes** : Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/10000 dilution

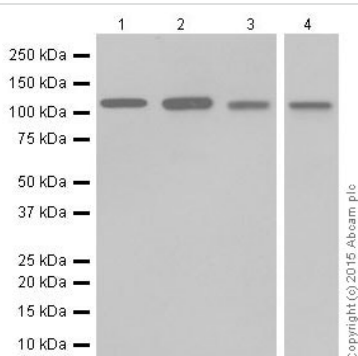
**Predicted band size:** 97 kDa

**Observed band size:** 110 kDa

**Exposure time:** 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The molecular weight observed is consistent with what have been described in the literature (PMID: 8606850, 17015635).



Western blot - Anti-NFkB p100/NFKB2 antibody [EPR18756] (ab191594)

**All lanes** : Anti-NFkB p100/NFKB2 antibody [EPR18756] (ab191594) at 1/5000 dilution

**Lane 1** : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

**Lane 2** : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

**Lane 3** : MCF7 (Human breast adenocarcinoma cell line) whole cell lysate

**Lane 4** : Daudi (Human Burkitt's lymphoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

### Secondary

**All lanes** : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

**Predicted band size:** 97 kDa

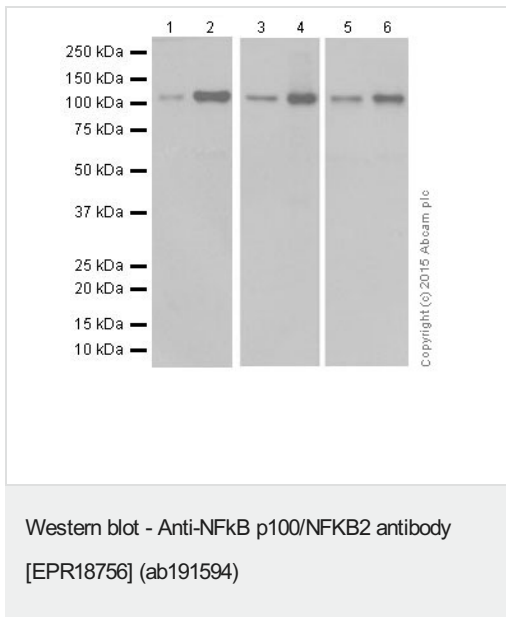
**Observed band size:** 110 kDa

Blocking/Dilution buffer: 5% NFDm/TBST.

Exposure time:

Lane 1, 2 & 3: 30 seconds; Lane 4: 5 seconds.

The molecular weight observed is consistent with what have been described in the literature (PMID: 18606850, 17015635).



**All lanes :** Anti-NFκB p100/NFKB2 antibody [EPR18756] (ab191594) at 1/1000 dilution

**Lane 1 :** Mouse heart lysate

**Lane 2 :** Mouse kidney lysate

**Lane 3 :** Rat brain lysate

**Lane 4 :** Rat heart lysate

**Lane 5 :** Rat kidney lysate

**Lane 6 :** Rat spleen lysate

Lysates/proteins at 10 μg per lane.

### Secondary

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

**Predicted band size:** 97 kDa

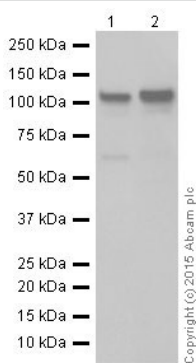
**Observed band size:** 110 kDa

Blocking/Dilution buffer: 5% NFDm/TBST.

Exposure time:

Lane 1 & 2: 3 minutes; Lane 3 & 4: 30 seconds; Lane 5 & 6: 5 seconds.

The molecular weight observed is consistent with what have been described in the literature (PMID: 18606850, 17015635).



Western blot - Anti-NFkB p100/NFKB2 antibody [EPR18756] (ab191594)

**All lanes** : Anti-NFkB p100/NFKB2 antibody [EPR18756] (ab191594) at 1/1000 dilution

**Lane 1** : C6 (Rat glial tumor cell line) whole cell lysate

**Lane 2** : NIH/3T3 (Mouse embryo fibroblast cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

### Secondary

**All lanes** : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

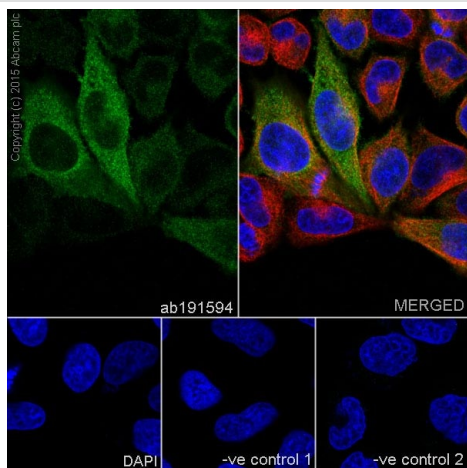
**Predicted band size:** 97 kDa

**Observed band size:** 110 kDa

**Exposure time:** 1 second

Blocking/Dilution buffer: 5% NFDM/TBST.

The molecular weight observed is consistent with what have been described in the literature (PMID: 18606850, 17015635).



Immunocytochemistry/ Immunofluorescence - Anti-NFkB p100/NFKB2 antibody [EPR18756] (ab191594)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cell line labeling NFkB p100/NFKB2 with ab191594 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

Confocal image showing cytoplasmic staining on HeLa cell line.

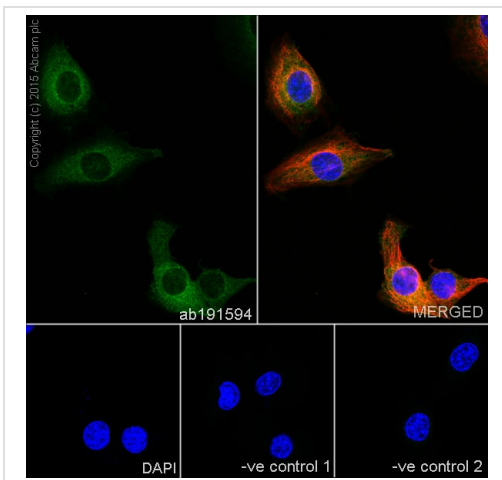
The nuclear counterstain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) at 1/1000 dilution and Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed (**ab150120**) at 1/1000 dilution (red).

The negative controls are as follows:-

-ve control 1: ab191594 at 1/250 dilution followed by **ab150120** at 1/1000 dilution.

-ve control 2: **ab7291** at 1/1000 dilution followed by **ab150077** at 1/1000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-NFKB p100/NFKB2 antibody [EPR18756] (ab191594)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (Mouse embryo fibroblast cell line) cell line labeling NFKB p100/NFKB2 with ab191594 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

Confocal image showing cytoplasmic staining on NIH/3T3 cell line.

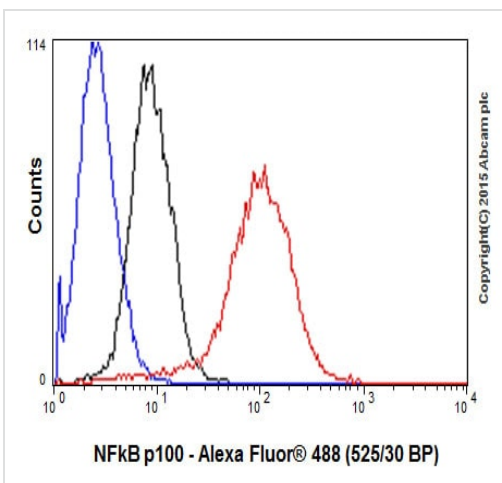
The nuclear counterstain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) at 1/1000 dilution and Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed (**ab150120**) at 1/1000 dilution (red).

The negative controls are as follows:-

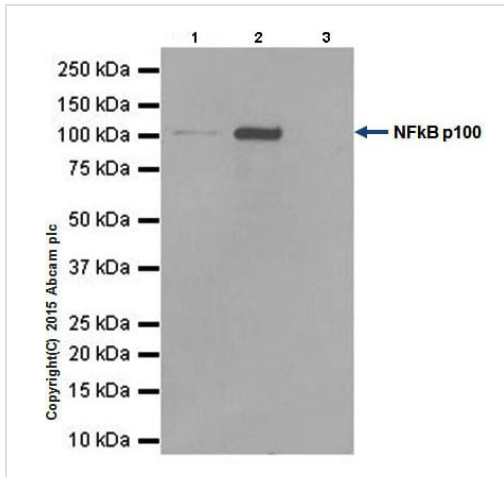
-ve control 1: ab191594 at 1/250 dilution followed by **ab150120** at 1/1000 dilution.

-ve control 2: **ab7291** at 1/1000 dilution followed by **ab150077** at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-NFKB p100/NFKB2 antibody [EPR18756] (ab191594)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HeLa (Human epithelial cell line from cervix adenocarcinoma) cell line labeling NFKB p100/NFKB2 with ab191594 at 1/60 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti Rabbit IgG (Alexa Fluor® 488) at 1/500 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-NFκB p100/NFKB2 antibody [EPR18756] (ab191594)

NFκB p100/NFKB2 was immunoprecipitated from 1mg of HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab191594 at 1/20 dilution.

Western blot was performed from the immunoprecipitate using ab191594 at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) ([ab131366](#)) was used for detection at 1/10000 dilution.

Lane 1: HeLa whole cell lysate, 10ug (Input).





Lane 2: ab191594 IP in HeLa whole cell lysate.

Lane 3: Rabbit IgG, monoclonal [EPR25A] - Isotype Control ([ab172730](#)) instead of ab191594 in HeLa whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDm/TBST.

Exposure time: 3 seconds.

Why choose a recombinant antibody?

 <b>Research with confidence</b> Consistent and reproducible results	 <b>Long-term and scalable supply</b> Recombinant technology
 <b>Success from the first experiment</b> Confirmed specificity	 <b>Ethical standards compliant</b> Animal-free production

Anti-NFκB p100/NFKB2 antibody [EPR18756] (ab191594)

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

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