

## Product datasheet

### Anti-NFkB p105 / p50 antibody [E381] - BSA and Azide free ab220803

KO VALIDATED

Recombinant

RabMAb

[37 References](#) [5 Images](#)

#### Overview

<b>Product name</b>	Anti-NFkB p105 / p50 antibody [E381] - BSA and Azide free
<b>Description</b>	Rabbit monoclonal [E381] to NFkB p105 / p50 - BSA and Azide free
<b>Host species</b>	Rabbit
<b>Specificity</b>	This antibody will detect both forms: p50 and p105.
<b>Tested applications</b>	<b>Suitable for:</b> WB, IHC-P <b>Unsuitable for:</b> Flow Cyt
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Rat, Human
<b>Immunogen</b>	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
<b>Positive control</b>	WB: HeLa, HAP1, Jurkat, MCF-7 and PC-12 cell lysates. IHC-P: Human prostate carcinoma and bladder carcinoma tissues.
<b>General notes</b>	<p>ab220803 is the carrier-free version of <a href="#">ab32360</a>.</p> <p>Our <b>carrier-free</b> 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.</p> <p>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.</p> <p>Use our <b>conjugation kits</b> for antibody conjugates that are ready-to-use in as little as 20 minutes with &lt;1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.</p> <p>This product is compatible with the Maxpar<sup>®</sup> Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar<sup>®</sup> is a trademark of Fluidigm Canada Inc.</p> <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>

Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to [RabMAb<sup>®</sup> patents](#).

## Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	Shipped at 4°C. Store at +4°C. Do Not Freeze.
<b>Storage buffer</b>	pH: 7.20 Constituent: PBS
<b>Carrier free</b>	Yes
<b>Purity</b>	Protein A purified
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	E381
<b>Isotype</b>	IgG

## Applications

**The Abpromise guarantee** Our [Abpromise guarantee](#) covers the use of ab220803 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
<b>WB</b>		Use at an assay dependent concentration. Detects a band of approximately 50, 105 kDa (predicted molecular weight: 50 kDa).
<b>IHC-P</b>		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See <a href="#">IHC antigen retrieval protocols</a> .

**Application notes** Is unsuitable for Flow Cyt.

## Target

**Function** NF-kappa-B is a pleiotropic transcription factor which is present in almost all cell types and is involved in 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 and the heterodimeric p65-p50 complex appears to be most abundant one. 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. NF-kappa-B heterodimeric p65-p50 and RelB-p50 complexes are transcriptional activators. The NF-kappa-B p50-p50 homodimer is a transcriptional repressor, but can act as a transcriptional activator when associated with BCL3. NFKB1 appears to have dual functions such as cytoplasmic retention of attached NF-kappa-B proteins by p105 and generation of p50 by a cotranslational processing. The proteasome-mediated process ensures the production of both p50 and p105 and preserves their independent function, although processing of NFKB1/p105 also appears to occur post-translationally. p50 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. In a complex with MAP3K8, NFKB1/p105 represses MAP3K8-induced MAPK signaling; active MAP3K8 is released by proteasome-dependent degradation of NFKB1/p105.

#### Sequence similarities

Contains 7 ANK repeats.  
Contains 1 death domain.  
Contains 1 RHD (Rel-like) domain.

#### Domain

The C-terminus of p105 might be involved in cytoplasmic retention, inhibition of DNA-binding, and transcription activation.  
Glycine-rich region (GRR) appears to be a critical element in the generation of p50.

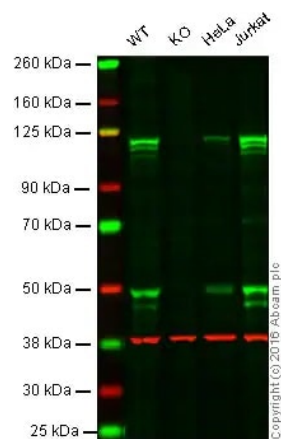
#### Post-translational modifications

While translation occurs, the particular unfolded structure after the GRR repeat promotes the generation of p50 making it an acceptable substrate for the proteasome. This process is known as cotranslational processing. The processed form is active and the unprocessed form acts as an inhibitor (I kappa B-like), being able to form cytosolic complexes with NF-kappa B, trapping it in the cytoplasm. Complete folding of the region downstream of the GRR repeat precludes processing.  
Phosphorylation at 'Ser-903' and 'Ser-907' primes p105 for proteolytic processing in response to TNF-alpha stimulation. Phosphorylation at 'Ser-927' and 'Ser-932' are required for BTRC/BTRCP-mediated proteolysis.  
Polyubiquitination seems to allow p105 processing.  
S-nitrosylation of Cys-61 affects DNA binding.

#### Cellular localization

Nucleus. Cytoplasm. Nuclear, but also found in the cytoplasm in an inactive form complexed to an inhibitor.

#### Images



Western blot - Anti-NFkB p105 / p50 antibody  
[E381] - BSA and Azide free (ab220803)

**All lanes :** Anti-NFkB p105 / p50 antibody [E381] ([ab32360](#))

**Lane 1 :** Wild-type HAP1 cell lysate

**Lane 2 :** NFkB p105 / p50 knockout HAP1 cell lysate

**Lane 3 :** HeLa cell lysate

**Lane 4 :** Jurkat cell lysate

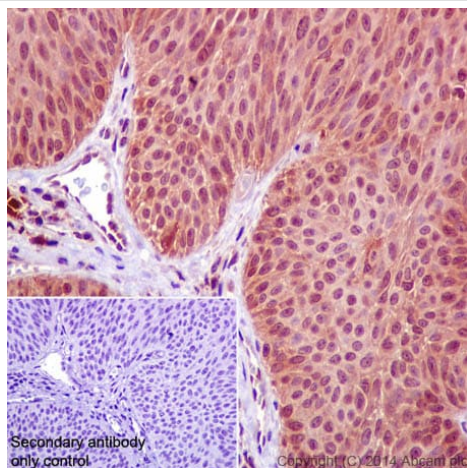
Lysates/proteins at 20 µg per lane.

**Predicted band size:** 50 kDa

**Lanes 1 - 4:** Merged signal (red and green). Green - [ab32360](#) observed at 120, 50 kDa. Red - loading control, [ab8245](#), observed at 37 kDa.

[ab32360](#) was shown to specifically react with NFkB p105 / p50 when NFkB p105 / p50 knockout samples were used. Wild-type and NFkB p105 / p50 knockout samples were subjected to SDS-PAGE. [ab32360](#) and [ab8245](#) (loading control to GAPDH) were diluted at 1/1000 and 1/10 000 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 hour at room temperature before imaging.

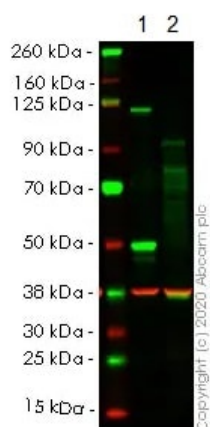
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([ab32360](#)).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NFkB p105 / p50 antibody [E381] - BSA and Azide free (ab220803)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human bladder carcinoma tissue labelling NFkB p105 / p50 with purified **ab32360** at 1/250. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab97051**, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32360**).



Western blot - Anti-NFkB p105 / p50 antibody [E381] - BSA and Azide free (ab220803)

**All lanes :** Anti-NFkB p105 / p50 antibody [E381] (**ab32360**) at 1/1000 dilution

**Lane 1 :** Wild-type HeLa cell lysate

**Lane 2 :** NFkB1 CRISPR/Cas9 edited HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 50 kDa

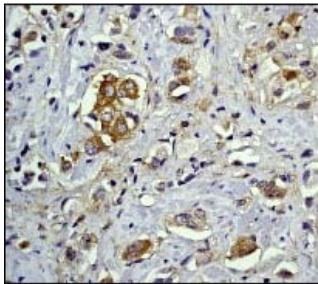
**Observed band size:** 105 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32360**).

**Lanes 1- 2:** Merged signal (red and green). Green - **ab32360** observed at 105 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

**ab32360** was shown to react with NFkB p105 / p50 in wild-type HeLa cells in western blot. The band observed in CRISPR/Cas9 edited cell line **ab264823** (CRISPR/Cas9 edited cell lysate **ab257003**) lane below 105kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type HeLa and NFkB1 CRISPR/Cas9 edited HeLa 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.  
**ab32360** 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.

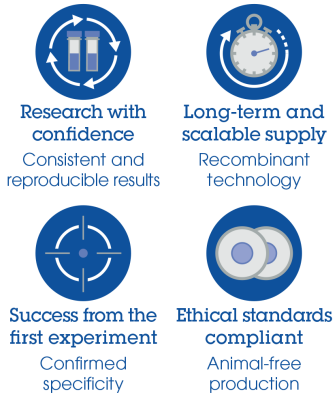


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NFKB p105 / p50 antibody [E381] - BSA and Azide free (ab220803)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human prostate carcinoma tissue labelling NFKB p105 / p50 with unpurified **ab32360** at a dilution of 1/250.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32360**).

#### Why choose a recombinant antibody?



Anti-NFKB p105 / p50 antibody [E381] - BSA and Azide free (ab220803)

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

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