abcam

Product datasheet

Anti-NFkB p105 / p50 antibody [E381] ab32360

敲除验证 重组 RabMAb

21 Abreviews 194 References 9 图像

概述

产品名称	Anti-NFkB p105 / p50**抗体**[E381]
描述	**兔单克隆抗体**[E381] to NFkB p105 / p50
宿主	Rabbit
特异性	This antibody will detect both forms: p50 and p105.
经测试应用	**适用于:** WB, IHC-P **不适用于:** Flow Cyt or IP
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: HeLa, MCF-7 and PC-12 cell lysates. IHC-P: human prostate carcinoma and bladder carcinoma tissues.
常规说明	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**.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
纯度	Protein A purified
克隆	单克隆

克隆编号	E381
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab32360于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB	★★★★★ (11)	1/1000 - 1/10000. Detects a band of approximately 50, 105 kDa (predicted molecular weight: 50 kDa).
IHC-P	★★★★☆ (5)	1/250 - 1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.

应用说明 Is unsuitable for Flow Cyt or IP.

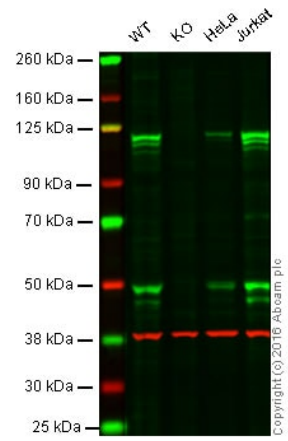
靶标

功能 NF-kappa-B is a pleiotropic transcription factor which is present in almost all cell types and is involved in many biological processed 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.

序列相似性 Contains 7 ANK repeats.
Contains 1 death domain.
Contains 1 RHD (Rel-like) domain.

结构域	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.
翻译后修饰	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.
细胞定位	Nucleus. Cytoplasm. Nuclear, but also found in the cytoplasm in an inactive form complexed to an inhibitor.

图片



Western blot - Anti-NFkB p105 / p50 antibody [E381] (ab32360)

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

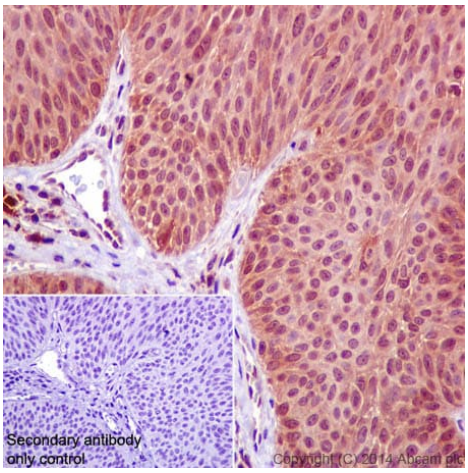
Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : NFkB p105 / p50 knockout HAP1 cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : Jurkat cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 50 kDa

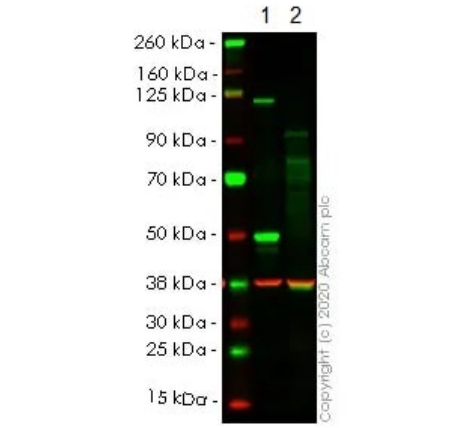
Lanes 1 - 4: Merged signal (red and green). Green - ab32360 observed at 120, 50 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

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 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NFkB p105 / p50 antibody [E381] (ab32360)

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.



Western blot - Anti-NFkB p105 / p50 antibody [E381] (ab32360)

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.

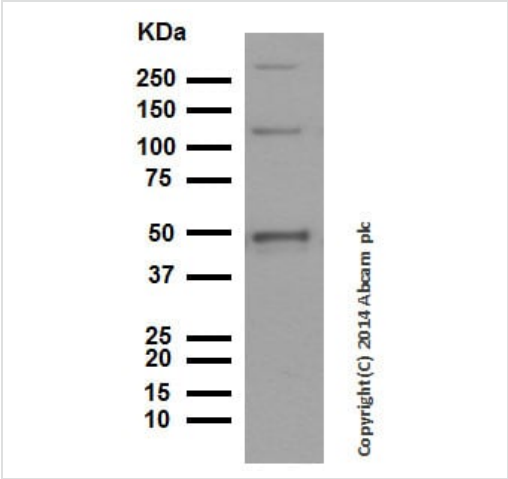
Performed under reducing conditions.

Predicted band size: 50 kDa
Observed band size: 105 kDa

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 HeLa 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.



Western blot - Anti-NFkB p105 / p50 antibody [E381] (ab32360)

Anti-NFkB p105 / p50 antibody [E381] (ab32360) at 1/10000 dilution (purified) + HeLa cell lysate at 20 µg

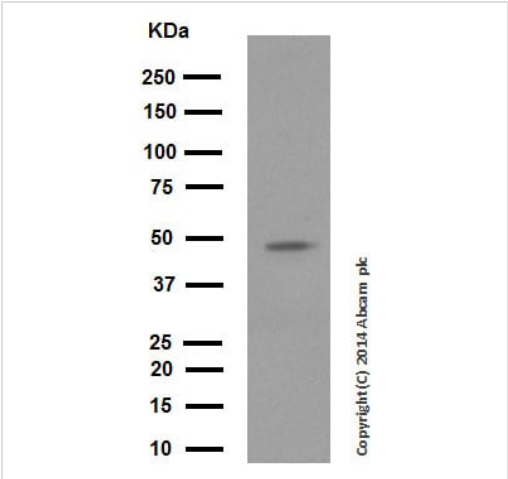
Secondary

Peroxidase conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 50 kDa

Observed band size: 105,50 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



Western blot - Anti-NFkB p105 / p50 antibody [E381] (ab32360)

Anti-NFkB p105 / p50 antibody [E381] (ab32360) at 1/50000 dilution (purified) + MCF-7 cell lysate at 10 µg

Secondary

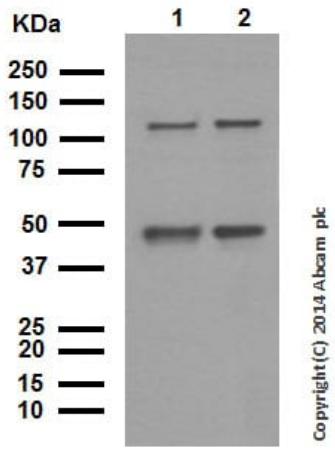
Peroxidase conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 50 kDa

Observed band size: 105 kDa

Additional bands at: 50 kDa (possible non-specific binding)

Blocking and dilution buffer: 5% NFDM/TBST.



Western blot - Anti-NFkB p105 / p50 antibody [E381] (ab32360)

All lanes : Anti-NFkB p105 / p50 antibody [E381] (ab32360) at 1/10000 dilution (purified)

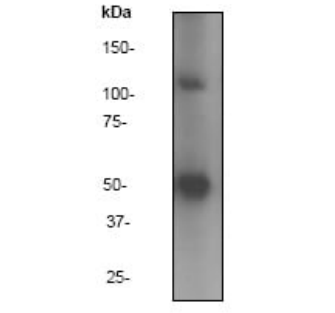
Lane 1 : PC-12 cell lysate
Lane 2 : NIH/3T3 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Peroxidase conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 50 kDa
Observed band size: 105 kDa
Additional bands at: 50 kDa (possible non-specific binding)

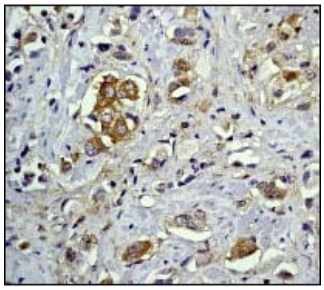
Blocking and dilution buffer: 5% NFDM/TBST.



Western blot - Anti-NFkB p105 / p50 antibody [E381] (ab32360)

Anti-NFkB p105 / p50 antibody [E381] (ab32360) at 1/5000 dilution (unpurified) + HeLa cell lysate

Predicted band size: 50 kDa
Observed band size: 105,50 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NFkB p105 / p50 antibody [E381] (ab32360)

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.

Anti-NFkB p105 / p50 antibody [E381] (ab32360)

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