Product name: Anti-NFkB p105 / p50 antibody [E381] ab32360

Description: Rabbit monoclonal [E381] to NFkB p105 / p50

Host species: Rabbit

Specificity: This antibody will detect both forms: p50 and p105.

Tested applications:
- Suitable for: WB, IHC-P
- Unsuitable for: Flow Cyt

Species reactivity: Reacts with: Mouse, Rat, Human

Immunogen: Synthetic peptide within NFkB p105/ p50 aa 300-400. The exact sequence is proprietary.

Positive control:
- WB: HeLa, MCF-7 and PC-12 cell lysates.
- IHC-P: human prostate carcinoma and bladder carcinoma tissues.

General notes:
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

This product is a recombinant rabbit monoclonal antibody.

Properties

Form: Liquid

Storage instructions:
- Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.

Storage buffer:
- pH: 7.20
- Preservative: 0.01% Sodium azide
- Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity: Protein A purified

Clonality: Monoclonal
Clone number: E381
Isotype: IgG

Applications

Our Abpromise guarantee covers the use of ab32360 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
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<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tbody>
<tr>
<td>WB</td>
<td>★★★★★</td>
<td>1/1000 - 1/10000. Detects a band of approximately 50, 105 kDa (predicted molecular weight: 50 kDa).</td>
</tr>
<tr>
<td>IHC-P</td>
<td>★★★★☆</td>
<td>1/250 - 1/500. See IHC antigen retrieval protocols.</td>
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</table>

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.

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**Images**

**Lane 1**: Wild-type HAP1 cell lysate (20 µg)
**Lane 2**: NFkB p105 / p50 knockout HAP1 cell lysate (20 µg)
**Lane 3**: HeLa cell lysate (20 µg)
**Lane 4**: Jurkat cell lysate (20 µg)

**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.

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

*why is the actual band size different from the predicted?*

Blocking and dilution buffer: 5% NFDM/TBST.

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.
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

*why is the actual band size different from the predicted?*

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

Blocking and dilution buffer: 5% NFDM/TBST.

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**All lanes:** Anti-NFkB p105 / p50 antibody [E381] (ab32360) at 1/100000 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

*why is the actual band size different from the predicted?*

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

Blocking and dilution buffer: 5% NFDM/TBST.
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

*why is the actual band size different from the predicted?*

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.

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

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