NF-kappa-B (p65) Antibody

Cat. No. CG1350

Applications:	**WB, IHC, IF**
Detected MW:	**60 kDa**
Species & Reactivity:	**Human, Mouse**
Isotype:	**Rabbit IgG**

BACKGROUND

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. The NF-kappa-B family includes five members, p65 (Rel-A), c-Rel, Rel-B, NF-kappa-B1 (p50 and its precursor p105), and NF-kappa-B2 (p52 and its precursor p100). In the inactive state, NF-kappa-B proteins are sequestered in the cytoplasm by IκB proteins (IkappaBalpha, IkappaBbeta, IkappaBepsilon, and IkappaBgamma). IkappaB inactivates NF-kappaB by masking the nuclear localization signals (NLS). Following stimulation, IκB kinase (IKK) complexes are activated to phosphorylate IkappaB proteins, which leads to proteasome-mediated degradation of IkappaB proteins. The released NF-kappa-B proteins translocate into the nucleus where they bind to kappaB sequences in the promoters of target genes to initiate transcription.[1] In general, activated NF-kappa-B dimers containing p65, c-Rel, or Rel-B can transactivate NF-kappa-B -dependent genes. In contrast, NF-kappa-B homodimers, p50/p50 and p52/p52, which lack transactivation domains, function primarily to inhibit NF-kappa-B -responsive genes. However, binding of p50/p50 or p52/p52 homodimers to B cell lymphoma 3 (Bcl-3), a transcriptional coactivator, confers the ability of these homodimers to induce NF-kappa-B responsive genes. Bcl-3 belongs to the IkappaB family and can interact with NF-kappa-B proteins through its ankyrin repeats. Unlike other IkappaB proteins, which are expressed in the cytoplasm and function as repressors of NF-kappa-B, Bcl-3 is predominately expressed in the nucleus and functions as an activator through interactions with p50 and p52 homodimers.[2] NF-kaapa-B1 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 NF-kappa-B1/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.[3]

In addition to regulation of NF-kappa-B activity through removal of IkappaB from NF-kappa-B/IκB complexes, NF-kappa-B activity is also regulated through modulation of its transcriptional function. Changes in NF-kappa-B transcriptional activity have been assigned to inducible phosphorylation of the p65 subunit at Ser276, Ser529, and Ser536 by a large variety of kinases in response to different stimuli.[4] Additionally, NF-kappa-B -dependent transcription requires multiple coactivators possessing histone acetyltransferase activity: CREB binding protein (CBP) and its homolog p300, p300/CBP-associated factor (P/CAF), SRC-1/NcoA-1, and TIF-2/GRIP-1/NcoA-2. Importantly, recruitment of CBP is enhanced by phosphorylation by the catalytic subunit of PKA (PKAc) of p65 at Ser276. More recently, other findings demonstrated a role for histone deacetylases (HDACs) as well. The first evidence came from the demonstration that inhibition of HDAC activity by trichostatin A (TSA) increases NF-kappa-B-dependent gene expression. It was next shown that NF-kappa-B interacts with distinct HDAC isoforms to negatively regulate gene expression, presumably through the deacetylation of histones and/or nonhistone proteins. Importantly, the phosphorylation status of p65 determines whether it associates with CBP/p300 or HDAC-1, ensuring that only signal-induced NF-kappa-B entering the nucleus can activate transcription.[5]

References:

1. Hayden, M.S. & Ghosh, S.: Cell 134:344-62, 2008
2. Dai, R. et al: J. Immunol.179:1776-83, 2007
3. Moorthy, A.K. et al: EMBO J. 25:1945-56, 2006
4. Vermeulen, L. et al: Biochem. Pharmacol. 64:673-90, 2002
5. Zhong, H. et al: Mol. Cell 9:625-36, 2002

TECHNICAL INFORMATION

Source:
NF-kappa-B (p65) Antibody is a rabbit antibody raised against a short peptide from C-terminal sequence of human Rel-A.

Specificity and Sensitivity:
This antibody detects endogenous NF-kappa-B (p65) proteins without cross-reactivity with other family members.

Storage Buffer: Anti-NF-κB p65 Antibody detects endogenous levels of total NF-κB p65 protein.

Storage:
Store at -20°C for at least one year. Store at 4°C for frequent use. Avoid repeated freeze-thaw cycles.

APPLICATIONS

Application:	*Dilution:
WB	1:500-1:1000
IP	n/d
IHC	1:50-1:100
ICC	n/d
FACS	n/d
IF	1:100-1:500

**Optimal dilutions must be determined by end user.*

CELL APPLICATIONS, INC.

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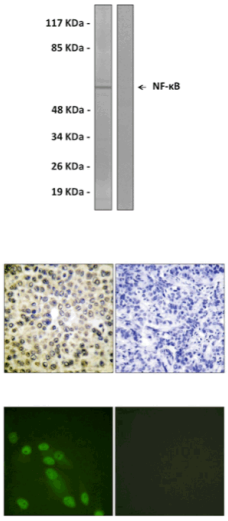
QUALITY CONTROL DATA



Top: Immunoblotting analysis of extracts from COS7 cells, treated with sorbitol 0.4M 24h, using Anti-NF-κB p65, C-Terminal antibody. The lane on the left was treated with the Anti-NF-κB p65, C-Terminal antibody. The lane on the right (negative control) was treated with both Anti-NF-κB p65, C-Terminal antibody and the synthesized immunogen peptide.
Middle: Immunohistochemistry analysis of paraffin-embedded human breast carcinoma tissue using Anti-NF-κB p65, C-Terminal antibody. Cells on the left were treated with the Anti-NF-κB p65, C-Terminal antibody. Cells on the right (negative control) were treated with both Anti-NF-κB p65, C-Terminal antibody and the synthesized immunogen peptide.
Bottom: Immunofluorescence of HeLa cells using Anti-NF-κB p65, C-Terminal antibody. Cells on the left were treated with the Anti-NF-κB p65, C-Terminal antibody. Cells on the right (negative control) were treated with both Anti-NF-κB p65, C-Terminal antibody and the synthesized immunogen peptide.