

BACKGROUND

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. 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 I κ B proteins (IkappaBalpha, IkappaBbeta, IkappaBepsilon, and IkappaBgamma). IkappaB inactivates NF-kappaB by masking the nuclear localization signals (NLS). Following stimulation, I κ B 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 κ B 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 I κ B family and can interact with NF-kappa-B proteins through its ankyrin repeats. Unlike other I κ B 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-kappa-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/I κ B 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:

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

TECHNICAL INFORMATION

Source:

NF-kappa-B (p65) Antibody is a mouse monoclonal antibody raised against purified recombinant human Rel-A fragments expressed in *E. coli*.

Specificity and Sensitivity:

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

Storage Buffer: PBS and 30% glycerol

Storage:

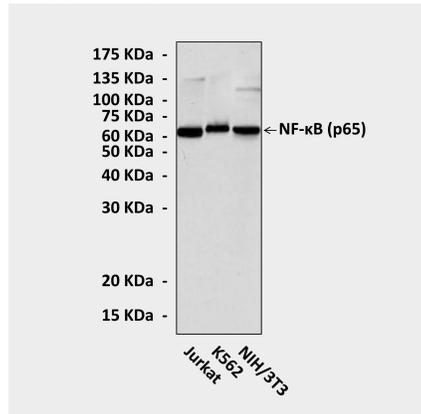
Store at -20°C for at least one year. Store at 4°C for frequent use. Avoid repeated freeze-thaw cycles.

APPLICATIONS

Application:	*Dilution:
WB	1:1,000
IP	1:50
IHC	1:200
ICC	n/d
FACS	n/d
*Optimal dilutions must be determined by end user.	



QUALITY CONTROL DATA



Western Blot detection of NF-kappa-B (p65) proteins in various cell lysates using NF-kappa-B (p65) Antibody.

