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 and 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 B proteins (IkappaBAlpha, IkappaBBeta, IkappaBepsilon, and IkappaBgamma). IkappaB inactivates NF-kappaB by masking the nuclear localization signals (NLS). Following stimulation, I 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 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-kappaB 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-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 kappaB 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/IkappaB 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:

TECHNICAL INFORMATION

Source:
c-Rel Antibody is a mouse monoclonal antibody raised against recombinant human c-Rel fragments expressed in E. coli.

Specificity and Sensitivity:
This antibody detects c-Rel proteins in various cell lysate.

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

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<tr>
<th>Application</th>
<th>Dilution</th>
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<tbody>
<tr>
<td>WB</td>
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</tr>
<tr>
<td>IP</td>
<td>1:25-50</td>
</tr>
<tr>
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<td>1:50-200</td>
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<tr>
<td>ICC</td>
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<tr>
<td>FACS</td>
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*Optimal dilutions must be determined by end user.
QUALITY CONTROL DATA

Top: Western blot detection of c-Rel proteins in various cell lysates using c-Rel Antibody. Middle: This antibody stains paraffin-embedded human liver cancer tissue in IHC analysis. Bottom: It also stains U251 cells in confocal immunofluorescent testing (c-Rel Antibody: Green; Actin filaments: Red).