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ProductInformation

Monoclonal Anti-NF-κB p65
Clone NF-12
Mouse Ascites Fluid

Product Number **N 8523**

Product Description

Monoclonal Anti-NF-κB p65 (mouse IgG1 isotype) is derived from the NF-12 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a BALB/c mouse immunized with a recombinant, mouse NF-κB p65 fragment (C-terminal 151 amino acids). The isotype is determined using Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

Monoclonal Anti-NF-κB p65 recognizes an epitope within the C-terminal region of the mouse NF-κB p65. The antibody is reactive in immunoblotting (65 kDa) and immunocytochemistry. Cross reactivity has been observed with human NF-κB p65.

Organisms must be able to respond rapidly and effectively to changes in their environment. Most types of signaling molecules induce cellular responses by binding to specific cell-surface receptors. These receptors respond to occupancy by undergoing structural or biochemical changes that can be transmitted to the interior of the cell. One of the most common responses to receptor ligation is the synthesis of new proteins through alteration of the pattern of gene expression. Consequently, the relatively few transcription factors that regulate inducible gene expression can be the targets for many distinct signal transduction pathways, triggered by a wide variety of stimuli.[1] One important transcription factor that plays a pivotal role in many cellular responses to environmental changes is NF-κB, a heterodimeric transcription factor composed of p50 (50 kDa) and p65 (65 kDa) subunits. NF-κB can be activated in many cell types and is thought to regulate a wide variety of genes.[2-4] An extensive set of genes with putative NF-κB-binding sites has been identified, and in many of these, the NF-κB sites appear crucial to the regulation of transcription. A wide range of stimuli lead to translocation of NF-κB from the cytoplasm to the nucleus, where it appears in an active form capable of binding decameric κB sequences motifs.[5] Putative cellular target genes are largely involved in the acute-phase response, inflammation, lymphocyte activation (specific and nonspecific immune responses), and cell growth and differentiation. These genes include cell-surface molecules involved in immune function such as immunoglobulin κ light chain, class I and II major histocompatability complex (MHC), and cytokines such as interleukin-1β (IL-1β), IL-2, IL-6, interferon-β (IFNβ), and tumor necrosis factor α (TNFα).[5] Under normal conditions, NF-κB is bound to an inhibitor protein, I-κB, which sequesters NF-κB in the cytosol. Activation of NF-κB involves its dissociation from I-κB followed by translocation of the p50-p65 heterodimer to the nucleus, where it directly binds to its cognate DNA sequences.[6,7] p50 and p65 are members of a larger NF-κB/Rel family of transcription factors, that in vertebrates includes at least five members: NFKB1 (p50 and its precursor, p105), NFKB2 (p52 and its precursor, p100), p65 (RelA), c-Rel (Rel), and RelB.[8] As dimers, all five proteins can form complexes with κB DNA sequence motifs, and all have been shown to affect transcription of κB reporter genes positively or negatively when assayed following transfection.[5] The high level of interest in Rel-based transcription factors is founded on their broad role in inducing and coordinately controlling genes of significant biomedical importance, such as those encoding inflammatory cytokines, chemokines, interferons, MHC proteins, growth factors, cell adhesion molecules, and viruses. Monoclonal antibodies, reacting specifically with the p65 subunit of NF-κB, are useful tools for the study of the physiological roles of NF-κB, and the control of different heterodimers within cells. They may also be used for understanding the heterodimers' tissue- and cell type-specific distribution and regulation within developing organisms, and the relationship of different heterodimeric complexes to the *in vivo* regulation of transcription of individual genes and regulatory genes networks.

Reagent
Monoclonal Anti-NF-κB p65 is supplied as ascites fluid with 15 mM sodium azide as a preservative.

Precautions and Disclaimer
Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability
For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile
By immunoblotting, a minimum working dilution of 1:1,000 is recommended using a whole cell extract of 3T3 mouse fibroblast cells.

Note: In order to obtain the best results in various techniques and preparation, we recommend determining optimal working dilution by titration.

References

1. May, M.J., and Ghosh, S., Immunol. Today, **19**, 80 (1998).
2. Sen, R., and Baltimore, D., Cell, **47**, 921 (1986).
3. Grilli, M., et al., Int. Rev. Cytol., **143**, 1 (1991).
4. Grimm, S., and Baeuerle, P.A., Biochem. J., **290**, 297 (1993).
5. Sha, W.C., et al., Cell, **80**, 321 (1995).
6. Baeuerle, P.A., and Baltimore, D., Cell, **87**, 13 (1996).
7. Thanos, D., and Maniatis, T., Cell, 80, 529 (1995).
8. Nolan, G.P., and Baltimore, D., Curr. Opin. Genet. Dev., 2, 211 (1992).

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