

Product Specification Sheet

**Recombinant NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) Protein p65**

□ **Cat.** NFKB651-R-5 Recombinant purified, human NFκB-p65 protein, active

**SIZE:** 5 ug

NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) is a protein complex that controls the transcription of DNA. NF-κB is found in almost all animal cell types and is involved in cellular responses to stimuli such as stress, cytokines, free radicals, ultraviolet irradiation, oxidized LDL, and bacterial or viral antigens. NF-κB plays a key role in regulating the immune response to infection (kappa light chains are critical components of immunoglobulins). Incorrect regulation of NF-κB has been linked to cancer, inflammatory and autoimmune diseases, septic shock, viral infection, and improper immune development. NF-κB has also been implicated in processes of synaptic plasticity and memory.

NF-κB was first discovered via its interaction with an 11-base pair sequence in the immunoglobulin light-chain enhancer in B cells. All proteins of the NF-κB family share a Rel homology domain in their N-terminus. A subfamily of NF-κB proteins, including RelA, RelB, and c-Rel, have a transactivation domain in their C-termini. In contrast, the NF-κB1 and NF-κB2 proteins are synthesized as large precursors, p105, and p100, which undergo processing to generate the mature NF-κB subunits, p50 and p52, respectively. The processing of p105 and p100 is mediated by the ubiquitin/proteasome pathway and involves selective degradation of their C-terminal region containing ankyrin repeats. Whereas the generation of p52 from p100 is a tightly-regulated process, p50 is produced from constitutive processing of p105.

There are five proteins in the mammalian NF-κB family:

Class	Protein	Aliases	Gene
I	NF-κB1	p105 → p50	NFKB1
	NF-κB2	p100 → p52	NFKB2
II	RelA	p65	RELA
	RelB		RELB
	c-Rel		REL

NF-κB is a major transcription factor that regulates genes responsible for both the innate and adaptive immune response. Upon activation of either the T- or B-cell receptor, NF-κB becomes activated through distinct signaling components. Upon ligation of the T-cell receptor, protein kinase Lck is recruited and phosphorylates the ITAMs of the CD3 cytoplasmic tail. ZAP70 is then recruited to the phosphorylated ITAMs and helps recruit LAT and PLC-γ, which causes activation of PKC. Through a cascade of phosphorylation events, the kinase complex is activated and NF-κB is able to enter the nucleus to upregulate genes involved in T-cell development, maturation, and proliferation.

Aberrant activation of NF-κB is frequently observed in many cancers. Moreover, suppression of NF-κB limits the proliferation of cancer cells. In addition, NF-κB is a key player in the inflammatory response. Hence methods of inhibiting NF-κB signaling has potential therapeutic application in cancer and inflammatory diseases. A new drug called denosumab acts to raise bone mineral density and reduce fracture rates in many patient subgroups by inhibiting RANKL.

**Source of Antigen**

Human NFκB p65 protein (protein accession #AAA36408 minus 14-aa deletion at the C-terminus) was expressed in E. coli and purified (>95%). This sequence had five point mutations compared to the p65 sequence listed under accession no. AAA36408: L159V, P180S, F309S, A439V and V462M. It is supplied lyophilized in Protein Dilution Buffer (20 mM Tris-HCl, 0.2 M NaCl, 2 mM MgCl<sub>2</sub> and 10% glycerol) and 1 M DTT. Protein is supplied at 100 ng/μl in Protein Dilution Buffer (see lot sp. conc on the vial) or lyophilized in the same buffer. Lyophilized proteins can be stored at -20°C or -80°C. Recombinant proteins in solution are temperature sensitive and must be stored at -80°C to prevent degradation. Avoid repeated freeze/thaw cycles and keep on ice when not in storage. Stable for at least 6-months.

**Suggested uses**

Recombinant NFκB p65 is suitable for western blot, gel shift and TransAM<sup>®</sup> assays. 100 ng is sufficient for gelshift and protein-protein interaction studies. The standard curve for TransAM NFκB p65 was generated using the range of 40 – 0.625 ng of protein. NOTE: The presence of Poly [d(I-C)] in buffers may affect protein functionality and should be avoided.

If using for TransAM assays, dilute the recombinant protein using the complete lysis buffer provided in the TransAM kit. For other applications using 1X protein buffer as mentioned above.

**General References:** Gilmore TD (2006) Oncogen 25, 6680-4; Brasier AR (2006) Cardiovascular Toxicol. 6, 111-130; Sen R (1986) Cell 46, 705-716; Meffert MK (2003) Nat. Neurosci. 6, 1072-1078

\*This product is for in vitro research use only.

**Related material available from ADI**

Catalog#	ProdDescription
NFKB501-R-5	Recombinant (E. coli, >95%) human NFκB-p50 protein, active

NFKB502-R-5	Recombinant purified (E. coli, >80%, GST-tag) human NFκB-p50 protein, active
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NFKB651-R-5	Recombinant purified, human NFκB-p650 protein, active
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