Technical Data Sheet

Purified Mouse anti-NF-κB p65 (pS529)

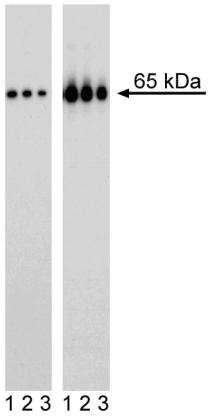
Product Information

Material Number:	**558393**
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	K10-895.12.50
Immunogen:	Phosphorylated Human NF-κB p65 Peptide
Isotype:	Mouse (BALB/c) IgG2b, κ
Reactivity:	QC testing: Human
Target MW:	65 kDa
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

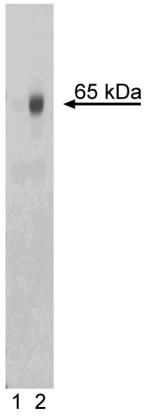
Description

Nuclear factor κB (NF-κB) is a ubiquitously expressed transcription factor that regulates the expression of 200-300 genes. It is crucial for basic cellular responses to stress and pathogens, such as proliferation, survival, development, and apoptosis. The most studied NF-κB complex consists of the p50 (also known as NF-κB1) and p65 (also known as REL-A) subunits, both containing a 300-amino acid region with homology to the Rel proto-oncogene product (RH domain). The RH domain contains motifs for dimerization, nuclear localization, and binding to specific DNA sequences. In addition to the RH domain, the p65 subunit contains the transactivation domain, which is responsible for the interaction with the inhibitor IκB and which contains phosphorylation sites. In most cell types, the p50/p65 heterodimer is located within the cytoplasm complexed to IκB. This complex prevents nuclear translocation and activity of NF-κB. In response to stimuli such as cytokines, LPS, DNA damage, and viral infections, IκB is phosphorylated at critical residues. This phosphorylation induces dissociation of the IκB/NF-κB complex, allowing the free heterodimeric NF-κB to translocate to the nucleus. Furthermore, optimal activation of NF-κB requires phosphorylation in the transactivation domain of p65. In the nucleus, activated NF-κB dimers bind to the κB sites within promoters and enhancers and function as transcriptional activators.

The K10-895.12.50 monoclonal antibody recognizes the phosphorylated serine 529 (pS529) in the transactivation domain of human NF-κB p65 subunit.



Western blot analysis of NF-κB p65 (pS529) in transformed human epithelioid carcinoma. *Lysates from control (left panel) and TNF-treated (Cat. No. 554618, right panel) HeLa cell line were probed with purified mouse anti-NF-κB p65 (pS529) monoclonal antibody at concentrations of 0.0125, 0.00625 and 0.00312 µg/ml (Lanes 1, 2, and 3, respectively). NF-κB p65 (pS529) is identified as a band of 65 kDa, which is upregulated in the treated cells.*



Western blot analysis of NF-κB p65 (pS529) in human peripheral blood mononuclear cells (PBMC). *Lysates from control ((lane 1) and PMA-treated (lane 2) PBMC were probed with purified mouse anti-NF-κB p65 (pS529) monoclonal antibody at 2.0 µg/ml. NF-κB p65 (pS529) is identified as a band of 65 kDa in the treated cells.*

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Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at 4°C.

The purified or conjugated mAb was characterized by flow cytometry (Flow) and western blot (WB) using these model systems:

Method	*Species*	*Cells*	*Treatment*	*Fixation*	*Perm buffer*	*Result*
Flow	Human	PBMC	PMA	Cytofix	Perm I, II, or III	Upregulated expression
		HeLa S3	TNF + Calyculin A	Cytofix	Perm III	Upregulated expression
WB	Human	PBMC	PMA			65-kDa band induced
		HeLa	TNF			65-kDa band induced
		HeLa	TNF + Lambda phosphatase			loss of signal

Application Notes

Application

Western blot	Routinely Tested
Intracellular staining (flow cytometry)	Tested During Development

Suggested Companion Products

Catalog Number	Name	Size	Clone
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

References

Natoli G, Saccani S, Bosisio D, Marazzi I. Interactions of NF-kappaB with chromatin: the art of being at the right place at the right time. *Nat Immunol.* 2005; 6(5):439-445.(Biology)

Siebenlist U, Brown K, Claudio E. Control of lymphocyte development by nuclear factor-kappaB. *Nat Rev Immunol.* 2005; 5:435-445.(Biology)

Viatour P, Merville M-P, Bours V, Chariot A. Phosphorylation of NF-kappaB and IkappaB proteins: implications in cancer and inflammation. *Trends Biochem Sci.* 2005; 30(1):43-52.(Biology)