Technical Data Sheet

Alexa Fluor® 647 Mouse anti-NF-κB p65 (pS529)

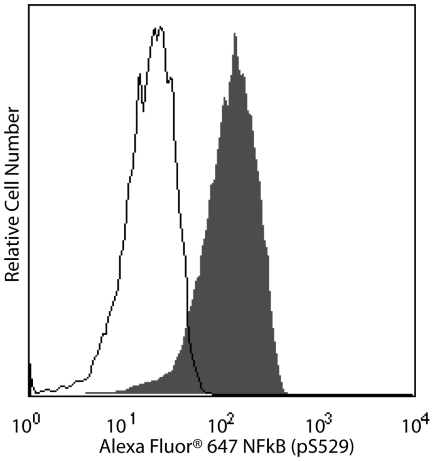
Product Information

Material Number:	**558422**
Alternate Name:	transcription factor p65, RELA, MGC131774, NFKB3
Size:	50 Tests
Vol. per Test:	20 µl
Clone:	K10-895.12.50
Immunogen:	Phosphorylated Human NF-κB p65 Peptide
Isotype:	Mouse (BALB/c) IgG2b, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤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.



Analysis of NFkB (pS529) in human peripheral blood lymphocytes. *Human peripheral blood mononuclear cells (PBMC) were either stimulated with 50 nM PMA (Sigma, P8139) for 15 minutes (shaded histogram) or unstimulated (open histogram). The PBMC were fixed with BD Cytofix™ buffer (Cat. No. 554655) for 10 minutes at 37°C, permeabilized (BD Phosflow™ Perm Buffer III, Cat. No. 558050) on ice for 30 minutes and then stained with Alexa Fluor® 647 Mouse anti-NF-κB p65 (pS529). For data analysis, lymphocytes were selected by scatter profile. Flow cytometry was performed on a BD FACSCanto™ II flow cytometry system.*

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.
The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

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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

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

This antibody conjugate is suitable for intracellular staining of human cell lines and peripheral blood mononuclear cells using BD Cytofix™ Fixation Buffer. Any of the three BD Phosflow™ permeabilization buffers may be used. For more information and suggested protocols, investigators are encouraged to visit http://www.bdbiosciences.com/research/ics/resources/index.jsp

Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 mL	(none)
557885	Perm/Wash Buffer I	125 mL	(none)
558052	Perm Buffer II	125 mL	(none)
558050	Perm Buffer III	125 mL	(none)
554656	Stain Buffer (FBS)	500 mL	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
4. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
5. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. 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.
8. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

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)

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