#3033 Store at –20°C

Phospho-NF-kB p65 (Ser536) (93H1) Rabbit mAb

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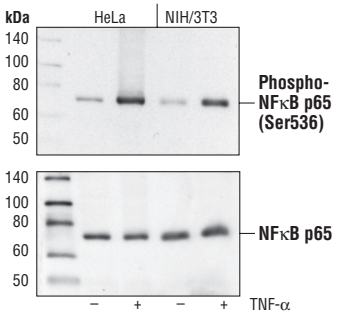
Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IF-IC, F Endogenous	H, M, R, Mk, Pg, Hm, (Dg)	65 kDa	Rabbit IgG**

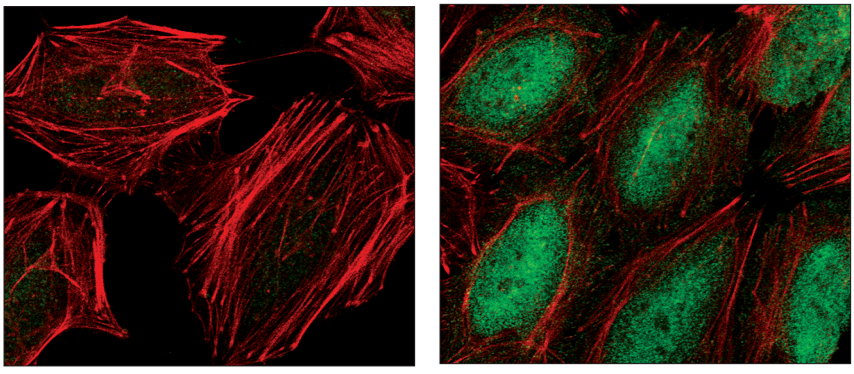
Background: Transcription factors of the nuclear factor κ B (NF-κB)/Rel family play a pivotal role in inflammatory and immune responses (1,2). There are five family members in mammals: RelA, c-Rel, RelB, NF-κB1 (p105/p50) and NF-κB2 (p100/p52). Both p105 and p100 are proteolytically processed by the proteasome to produce p50 and p52, respectively. Rel proteins bind p50 and p52 to form dimeric complexes that bind DNA and regulate transcription. In unstimulated cells, NF-κB is sequestered in the cytoplasm by IκB inhibitory proteins (3-5). NF-κB-activating agents can induce the phosphorylation of IκB proteins, targeting them for rapid degradation through an ubiquitin-proteasome pathway and releasing NF-κB to enter the nucleus where it regulates gene expression (6-8). NIK and IKKα (IKK1) regulate the phosphorylation and processing of NF-κB2 (p100) to produce p52, which is then translocated to the nucleus (9-11).

Specificity/Sensitivity: Phospho-NF-kappaB p65 (Ser536) (93H1) Rabbit mAb detects NF-κB p65 only when phosphorylated at Ser536. It does not cross-react with the p50 subunit or other related proteins.



Western blot analysis of extracts from HeLa and NIH/3T3 cells, untreated or TNF-a treated (#2169, 20 ng/ml for 5 minutes), using Phospho-NF-kB p65 (Ser536) (93H1) Rabbit mAb (upper) or NF-kB p65 Antibody #3034 (lower).

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser536 of human NF-κB p65.



Confocal immunofluorescent analysis of HeLa cells, untreated (left) and TNF-α treated (#8902 at 20 ng/ml for 20 min, right), using Phospho-NF-κB p65 (Ser536) (93H1) Rabbit mAb (green). Actin filaments have been labeled with Alexa Fluor® phalloidin 555 (red).

IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.

Entrez-Gene ID #5970
UniProt ID #Q04206

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibody.*

***Species cross-reactivity is determined by western blot.**

****Anti-rabbit secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

Western Blotting	1:1000
Immunoprecipitation	1:50
Immunofluorescence (IF-IC)	1:100
Flow Cytometry	1:1600

For product specific protocols please see the web page for this product at www.cellsignal.com.

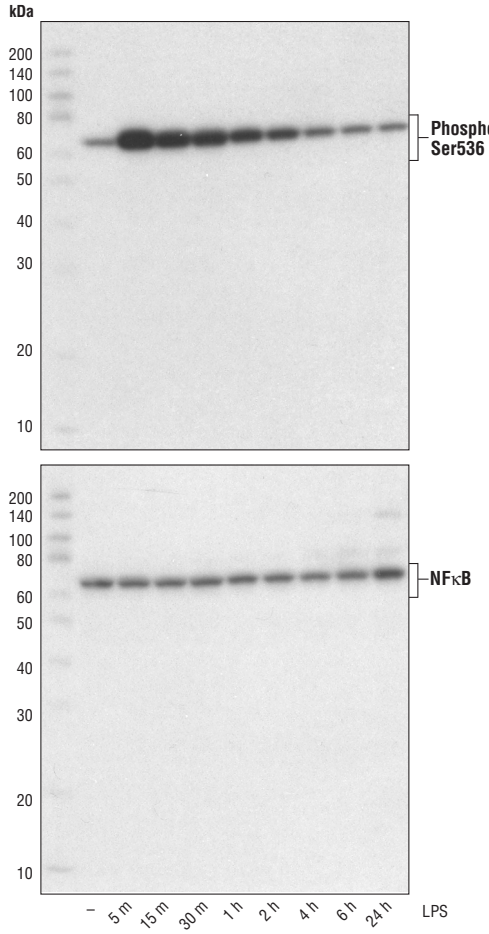
Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

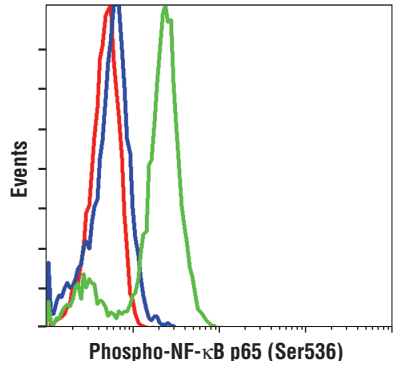
(1) Baeuerle, P.A. and Henkel, T. (1994) *Annu. Rev. Immunol.* 12, 141–179.
(2) Baeuerle, P.A. and Baltimore, D. (1996) *Cell* 87, 13–20.
(3) Haskill, S. et al. (1991) *Cell* 65, 1281–1289.
(4) Thompson, J.E. et al. (1995) *Cell* 80, 573–582.
(5) Whiteside, S.T. et al. (1997) *EMBO J.* 16, 1413–1426.
(6) Traenckner, E.B. et al. (1995) *EMBO J.* 14, 2876–2883.
(7) Scherer, D.C. et al. (1995) *Proc. Natl. Acad. Sci. USA* 92, 11259–11263.
(8) Chen, Z.J. et al. (1996) *Cell* 84, 853–862.
(9) Senftleben, U. et al. (2001) *Science* 293, 1495–1499.
(10) Coope, H.J. et al. (2002) *EMBO J.* 21, 5375–5385.
(11) Xiao, G. et al. (2001) *Mol. Cell* 7, 401–409.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



Western blot analysis of extracts from THP-1 cells, differentiated with TPA (#9905, 80 nM for 24h) and treated with 1 µg/ml LPS for the indicated times, using Phospho-NF-κB p65 (Ser536) (93H1) Rabbit mAb (upper) and NF-κB p65 (C22B4) Rabbit mAb #4764 (lower).



Flow cytometric analysis of HeLa cells, untreated (blue) or TNF-a-treated (green), using Phospho-NF-kB p65 (Ser536) (93H1) Rabbit mAb compared to a nonspecific negative control antibody (red).