

# NF- $\kappa$ B p65 Antibody Sampler Kit



**Orders:** 877-616-CELL (2355)  
orders@cellsignal.com

**Support:** 877-678-TECH (8324)

**Web:** info@cellsignal.com  
cellsignal.com

1 Kit (5 x 20 microliters)

3 Trask Lane | Danvers | Massachusetts | 01923 | USA

**For Research Use Only. Not for Use in Diagnostic Procedures.**

Product Includes	Product #	Quantity	Mol. Wt	Isotype/Source
Phospho-NF- $\kappa$ B p65 (Ser536) (93H1) Rabbit mAb	3033	20 $\mu$ l	65 kDa	Rabbit IgG
Acetyl-NF- $\kappa$ B p65 (Lys310) (D2S3J) Rabbit mAb	12629	20 $\mu$ l	65 kDa	Rabbit IgG
NF- $\kappa$ B p65 (L8F6) Mouse mAb	6956	20 $\mu$ l	65 kDa	Mouse IgG2b
NF- $\kappa$ B p65 (D14E12) XP <sup>®</sup> Rabbit mAb	8242	20 $\mu$ l	65 kDa	Rabbit IgG
Phospho-NF- $\kappa$ B p65 (Ser468) Antibody	3039	20 $\mu$ l	65 kDa	Rabbit
Anti-rabbit IgG, HRP-linked Antibody	7074	100 $\mu$ l		Goat
Anti-mouse IgG, HRP-linked Antibody	7076	100 $\mu$ l		Horse

Please visit [cellsignal.com](http://cellsignal.com) for individual component applications, species cross-reactivity, dilutions, protocols, and additional product information.

## Description

The NF- $\kappa$ B p65 Antibody Sampler Kit contains reagents to examine NF- $\kappa$ B p65/RelA phosphorylation at Ser468 and Ser536; acetylation at Lys310; and total p65 levels.

## Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100  $\mu$ g/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

## Background

Transcription factors of the nuclear factor  $\kappa$ B (NF- $\kappa$ B)/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- $\kappa$ B1 (p105/p50), and NF- $\kappa$ B2 (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- $\kappa$ B is sequestered in the cytoplasm by I $\kappa$ B inhibitory proteins (3-5). NF- $\kappa$ B-activating agents can induce the phosphorylation of I $\kappa$ B proteins, targeting them for rapid degradation through the ubiquitin-proteasome pathway and releasing NF- $\kappa$ B to enter the nucleus where it regulates gene expression (6-8). NIK and IKK $\alpha$  (IKK1) regulate the phosphorylation and processing of NF- $\kappa$ B2 (p100) to produce p52, which translocates to the nucleus (9-11).

RelA/p65 is a subunit of the NF- $\kappa$ B transcription complex, which plays a crucial role in inflammatory and immune responses. The complex is composed of various homodimeric and heterodimeric Rel family member combinations, the activity of which is modulated by post-translational modifications including phosphorylation and acetylation. p65 phosphorylation by PKA and/or MSK1 at Ser276 allows for increased interaction with the transcriptional coactivator p300/CBP to enhance transcriptional activity. NF- $\kappa$ B dimer assembly with I $\kappa$ B, as well as its DNA binding and transcriptional activities, are regulated by p300/CBP acetyltransferases that principally target Lys218, Lys221 and Lys310 (12-14). This process is reciprocally regulated by histone deacetylases (HDACs); several HDAC inhibitors have been shown to activate NF- $\kappa$ B (12-14). T-cell co-stimulation and Calyculin A have both been shown to increase Ser468 phosphorylation (15,16). IKK $\beta$  (but not IKK $\alpha$ ) and GSK-3 $\beta$  both target this site (16,17), which appears to have a negative regulatory role not involving inhibition of nuclear translocation after TNF- $\alpha$  or IL-1 $\beta$  stimulation (17). p65 phosphorylation at Ser536 regulates activation, nuclear localization, protein-protein interactions, transcriptional activity, and apoptosis (18-22).

## Background References

- Baeuerle, P.A. and Henkel, T. (1994) *Annu Rev Immunol* 12, 141-79.
- Baeuerle, P.A. and Baltimore, D. (1996) *Cell* 87, 13-20.
- Haskill, S. et al. (1991) *Cell* 65, 1281-9.
- Thompson, J.E. et al. (1995) *Cell* 80, 573-82.
- Whiteside, S.T. et al. (1997) *EMBO J* 16, 1413-26.
- Traenckner, E.B. et al. (1995) *EMBO J* 14, 2876-83.
- Scherer, D.C. et al. (1995) *Proc Natl Acad Sci USA* 92, 11259-63.
- Chen, Z.J. et al. (1996) *Cell* 84, 853-62.
- Senftleben, U. et al. (2001) *Science* 293, 1495-9.
- Coope, H.J. et al. (2002) *EMBO J* 21, 5375-85.
- Xiao, G. et al. (2001) *Mol Cell* 7, 401-9.
- Ashburner, B.P. et al. (2001) *Mol Cell Biol* 21, 7065-77.
- Mayo, M.W. et al. (2003) *J Biol Chem* 278, 18980-9.

14. Chen, L.F. et al. (2002) *EMBO J* 21, 6539-48.
  15. Mattioli, I. et al. (2004) *Blood* 104, 3302-4.
  16. Buss, H. et al. (2004) *J Biol Chem* 279, 49571-4.
  17. Schwabe, R.F. and Sakurai, H. (2005) *FASEB J* 19, 1758-60.
  18. Doyle, S.L. et al. (2005) *J Biol Chem* 280, 23496-501.
  19. Sasaki, C.Y. et al. (2005) *J Biol Chem* 280, 34538-47.
  20. Mattioli, I. et al. (2004) *J Immunol* 172, 6336-44.
  21. Bae, J.S. et al. (2003) *Biochem Biophys Res Commun* 305, 1094-8.
  22. Buss, H. et al. (2004) *J Biol Chem* 279, 55633-43.
- 

## Trademarks and Patents

Cell Signaling Technology is a trademark of Cell Signaling Technology, Inc.

All other trademarks are the property of their respective owners. Visit [cellsignal.com/trademarks](http://cellsignal.com/trademarks) for more information.

## Limited Uses

Except as otherwise expressly agreed in a writing signed by a legally authorized representative of CST, the following terms apply to Products provided by CST, its affiliates or its distributors. Any Customer's terms and conditions that are in addition to, or different from, those contained herein, unless separately accepted in writing by a legally authorized representative of CST, are rejected and are of no force or effect.

Products are labeled with For Research Use Only or a similar labeling statement and have not been approved, cleared, or licensed by the FDA or other regulatory foreign or domestic entity, for any purpose. Customer shall not use any Product for any diagnostic or therapeutic purpose, or otherwise in any manner that conflicts with its labeling statement. Products sold or licensed by CST are provided for Customer as the end-user and solely for research and development uses. Any use of Product for diagnostic, prophylactic or therapeutic purposes, or any purchase of Product for resale (alone or as a component) or other commercial purpose, requires a separate license from CST. Customer shall (a) not sell, license, loan, donate or otherwise transfer or make available any Product to any third party, whether alone or in combination with other materials, or use the Products to manufacture any commercial products, (b) not copy, modify, reverse engineer, decompile, disassemble or otherwise attempt to discover the underlying structure or technology of the Products, or use the Products for the purpose of developing any products or services that would compete with CST products or services, (c) not alter or remove from the Products any trademarks, trade names, logos, patent or copyright notices or markings, (d) use the Products solely in accordance with CST Product Terms of Sale and any applicable documentation, and (e) comply with any license, terms of service or similar agreement with respect to any third party products or services used by Customer in connection with the Products.