

**NF- $\kappa$ B Pathway Antibody Sampler Kit II**

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1 Kit (9 x 20 microliters)

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

Product Includes	Product #	Quantity	Mol. Wt	Isotype/Source
IKK $\alpha$ (D3W6N) Rabbit mAb	61294	20 $\mu$ l	85 kDa	Rabbit IgG
IKK $\beta$ (D30C6) Rabbit mAb	8943	20 $\mu$ l	87 kDa	Rabbit IgG
Phospho-IKK $\alpha$ / $\beta$ (Ser176/180) (16A6) Rabbit mAb	2697	20 $\mu$ l	85 IKK-alpha 87 IKK-beta kDa	Rabbit IgG
I $\kappa$ B $\alpha$ (44D4) Rabbit mAb	4812	20 $\mu$ l	39 kDa	Rabbit IgG
Phospho-I $\kappa$ B $\alpha$ (Ser32) (14D4) Rabbit mAb	2859	20 $\mu$ l	40 kDa	Rabbit IgG
NF- $\kappa$ B p65 (D14E12) XP <sup>®</sup> Rabbit mAb	8242	20 $\mu$ l	65 kDa	Rabbit IgG
Phospho-NF- $\kappa$ B p65 (Ser536) (93H1) Rabbit mAb	3033	20 $\mu$ l	65 kDa	Rabbit IgG
NF- $\kappa$ B1 p105/p50 (D4P4D) Rabbit mAb	13586	20 $\mu$ l	50 Active form. 120 Precursor kDa	Rabbit IgG
Phospho-NF- $\kappa$ B p65 (Ser529) Antibody	96874	20 $\mu$ l	65 kDa	Rabbit
Anti-rabbit IgG, HRP-linked Antibody	7074	100 $\mu$ l		Goat

Please visit cellsignal.com for individual component applications, species cross-reactivity, dilutions, protocols, and additional product information.

**Description**

The NF- $\kappa$ B Pathway Antibody Sampler Kit II contains reagents to examine the activation state and total protein levels of key proteins in the NF- $\kappa$ B pathway: IKK $\alpha$ , IKK $\beta$ , NF- $\kappa$ B p65/RelA, and I $\kappa$ B $\alpha$ . The kit includes enough antibodies to perform two western blot experiments with each primary antibody.

**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 antibodies.*

**Background**

The transcriptional nuclear factor  $\kappa$ B (NF- $\kappa$ B)/Rel transcription factors are present in the cytosol in an inactive state, complexed with the inhibitory I $\kappa$ B proteins. Activation occurs via phosphorylation of I $\kappa$ B $\alpha$  at Ser32 and Ser36, resulting in the ubiquitin-mediated proteasome-dependent degradation of I $\kappa$ B $\alpha$  and the release and nuclear translocation of active NF- $\kappa$ B dimers. The regulation of I $\kappa$ B $\beta$  and I $\kappa$ B $\epsilon$  is similar to that of I $\kappa$ B $\alpha$ , however, the phosphorylation and degradation of these proteins occurs with much slower kinetics. Phosphorylation of I $\kappa$ B $\beta$  occurs at Ser/Thr19 and Ser23, while I $\kappa$ B $\epsilon$  can be phosphorylated at Ser18 and Ser22. The key regulatory step in this pathway involves activation of a high molecular weight I $\kappa$ B kinase (IKK) complex, consisting of three tightly associated IKK subunits. IKK $\alpha$  and IKK $\beta$  serve as the catalytic subunits of the kinase. Activation of IKK depends on phosphorylation at Ser177 and Ser181 in the activation loop of IKK $\beta$  (176 and 180 in IKK $\alpha$ ). NF- $\kappa$ B-inducing kinase (NIK), TANK-binding kinase 1 (TBK1), and its homolog IKK $\epsilon$  (IKKi), phosphorylate and activate IKK $\alpha$  and IKK $\beta$ .

The NF- $\kappa$ B family of transcription factors is comprised of five proteins in mammals, p65/RelA, c-Rel, RelB, NF- $\kappa$ B1 (p105/p50), and NF- $\kappa$ B2 (p100/p52). p105 and p100 are proteolytically processed to produce p50 and p52, respectively. The 50 kDa active form is produced through proteolytic processing following IKK-mediated phosphorylation of p105 at multiple sites (Ser922, 924, 928, and 933), while p100's processing to p52 is induced by phosphorylation of Ser864 and Ser868. The p50 and p52 products form dimeric complexes with Rel proteins, which are then able to bind DNA and regulate transcription. Phosphorylation of p65/RelA at Ser276 by PKA C and MSK1 enhances transcriptional activity. p65 phosphorylation at Ser536 regulates activation, nuclear localization, protein-protein interactions, and transcriptional activity. PMA-induced NF- $\kappa$ B transcriptional activity is dependent on the region of p65 containing the potential phosphorylation sites Ser457, Thr458, Thr464, and Ser468. Phosphorylation of Ser468 by GSK-3 $\beta$  inhibits basal p65 activity.

**Background References**

1. Yamamoto, Y. and Gaynor, R.B. (2004) *Trends Biochem. Sci.* 29, 72-79.
2. Ghosh, S. and Karin, M. (2002) *Cell* 109, S81-S96.
3. Viatour, P. et al. (2005) *Trends Biochem. Sci.* 30, 43-52.
4. Ho, C.M. et al. (2016) *PLoS One* 11, e0150339.
5. Beyaz, S. et al. (2016) *Nature* 531, 53-8.

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