Revision 4

Store at -20°C

#9936

NF-κB Pathway Antibody Sampler Kit

1 Kit (7 x 20 microliters)

Cell Signaling TECHNOLOGY®

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

Product Includes	Product #	Quantity	Mol. Wt	Isotype/Source
IKKα (3G12) Mouse mAb	11930	20 µl	85 kDa	Mouse IgG1
IKKβ (D30C6) Rabbit mAb	8943	20 µl	87 kDa	Rabbit IgG
Phospho-IKKα/β (Ser176/180) (16A6) Rabbit mAb	2697	20 µl	85 IKK-alpha 87 IKK-beta kDa	Rabbit IgG
Phospho-NF-κB p65 (Ser536) (93H1) Rabbit mAb	3033	20 µl	65 kDa	Rabbit IgG
IκBα (L35A5) Mouse mAb (Amino-terminal Antigen)	4814	20 µl	39 kDa	Mouse IgG1
Phospho-IκBα (Ser32) (14D4) Rabbit mAb	2859	20 µl	40 kDa	Rabbit IgG
NF-κB p65 (D14E12) XP® Rabbit mAb	8242	20 µl	65 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat
Anti-mouse IgG, HRP-linked Antibody	7076	100 µl		Horse

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

Description

The NF-κB Pathway Antibody Sampler Kit contains reagents to examine the activation state and total protein levels of key proteins in the NF-κB pathway: IKKα, IKKβ, NF-κB p65/RelA, and IκBα. The kit contains enough primary and secondary antibodies to perform two Western blot experiments per primary antibody.

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.

Background

The transcriptional nuclear factor κB (NF-κB)/Rel transcription factors are present in the cytosol in an inactive state, complexed with the inhibitory IκB proteins. Activation occurs via phosphorylation of IκBα at Ser32 and Ser36, resulting in the ubiquitin-mediated proteasome-dependent degradation of IκBα and the release and nuclear translocation of active NF-κB dimers. The regulation of IκBβ and IκBε is similar to that of IκBα, however, the phosphorylation and degradation of these proteins occurs with much slower kinetics. Phosphorylation of IκBβ occurs at Ser/Thr19 and Ser23, while IκBε can be phosphorylated at Ser18 and Ser22. The key regulatory step in this pathway involves activation of a high molecular weight IkappaB kinase (IKK) complex, consisting of three tightly associated IKK subunits. IKKα and IKKβ serve as the catalytic subunits of the kinase. Activation of IKK depends on phosphorylation at Ser177 and Ser181 in the activation loop of IKKβ (176 and 180 in IKKα). NF-κB-inducing kinase (NIK), TANK-binding kinase 1 (TBK1), and its homolog IKKε (IKKi), phosphorylate and activate IKKα and IKKβ.
The NF-κB family of transcription factors is comprised of five proteins in mammals, p65/RelA, c-Rel, RelB, NF-κB1 (p105/p50) and NF-κB2 (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-κB transcriptional activity is dependent on the region of p65 containing the potential phosphorylation sites Ser457, Thr458, Thr464 and Ser468. Phosphorylation of Ser468 by GSK-3β 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. et al. (2016) PLOS One 10,1-22.
5. Beyaz S. et al. (2016) Nature 531, 53-58.

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