$I\kappa B\alpha$ (Tyr-305), phospho-specific

Background:

The NF- κ B/Rel transcription factors are present in the cytosol in an inactive state complexed with the inhibitory IkB proteins. Activation of $I\kappa B\alpha$ occurs through both serine and tyrosine phosphorylation events. Activation through phosphorylation at Ser-32 and Ser-36 is followed by proteasome-mediated degradation, resulting in the release and nuclear translocation of active NF- κ B. This pathway of I κ B α regulation occurs in response to various NF- κ B-activating agents, such as TNF α , interleukins, LPS, and irradiation. An alternative pathway for $I\kappa B\alpha$ regulation occurs through tyrosine phosphorylation of Tyr-42 and Tyr-305. Tyr-42 is phosphorylated in response to oxidative stress and growth factors. This phosphorylation can lead to degradation of $I\kappa B\alpha$ and NF- κB -activation. In contrast, Tyr-305 phosphorylation by c-Abl has been implicated in $I\kappa B\alpha$ nuclear translocation and inhibition of NF-kB-activation. Thus, tyrosine phosphorylation of $I\kappa B\alpha$ may be an important regulatory mechanism in NF-kB signaling.

References

Bui, N.T. et al. (2001) J Cell Biol 152(4):753. Finco, T.S. et al. (1994) Proc. Natl. Acad. Sci. USA 91:11884. Waris et al. (2003) J Biol Chem 278(42):40778.

Immunogen:

 $I\kappa B\alpha$ (Tyr-305) synthetic peptide (coupled to KLH) corresponding to amino acid residues around tyrosine 305 of human IkBa. This peptide sequence has low homology to other IkB proteins.

Applications:

WB	1:500
ELISA	1:2000

End user should determine optimal dilution for their particular applications and experiments. Western blot membranes were incubated with diluted antibody in 5% non-fat milk, PBS, 0.04% Tween20 for 1hour at room temperature

Related Products:

IP1031 IκBα (Tyr-42), phospho-specific Rabbit Polyclonal

IP1861 IkBa (C-terminus) Rabbit Polyclonal

IX1035 phospho-IκBα (Tyr-42) Peptide

IX1045 phospho-IkBa (Tyr-305) Peptide

AL9401 A431 Pervanadate Ctrl Lysate

AL9501 A431 + Pervanadate Lysate

Buffer and Storage:

Rabbit polyclonal, affinity-purified antibody is supplied in 100µl phosphate-buffered saline, 50% glycerol, 1 mg/ml BSA, and 0.05% sodium azide. Store at -20oC. Do not aliquot. Stable for 1 year.

Specificity:

This antibody was cross-adsorbed to phospho-tyrosine coupled to agarose then affinity purified using phospho- $I\kappa B\alpha$ (Tyr-305) peptide (without carrier). The antibody detects a 38 kDa* protein on SDS-PAGE immunoblots of A431 and Jurkat cells treated with pervanadate, but does not detect this band in control cells.

*All molecular weights (MW) are confirmed by comparison to Bio-Rad Rainbow Markers and to western blot mobilities of known proteins with similar MW.

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Western blot analysis of A431 cells treated with pervanadate (1 mM) for 30 min. Blots were probed with anti-I κ B α (lane 1), anti-I κ B α (Tyr-42) (IP1031; lanes 2-5), or anti-IkBa (Tyr-305) (IP1041; lanes 6-9). The latter two antibodies were used in the presence of no blocking peptide (lane 2 & 6), phospho-IkBa (Tyr-42) peptide (lane 3 & 8), phospho-I κ B α (Tyr-305) peptide (lane 4 & 7), or BSA conjugated to phospho-tyrosine (lane 5 & 9). Peptides and BSA-pTyr were used at 1 µg/ml.





Cat. # IP1041 Host Rabbit Polyclonal Size 100 µl