

# I $\kappa$ B $\alpha$ (Tyr-42), phospho-specific

Cat. # IP1031

Host Rabbit Polyclonal

Size 100  $\mu$ l

## Background:

The NF- $\kappa$ B/Rel transcription factors are present in the cytosol in an inactive state complexed with the inhibitory I $\kappa$ B 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- $\kappa$ B. This pathway of I $\kappa$ B $\alpha$  regulation occurs in response to various NF- $\kappa$ B-activating agents, such as TNF $\alpha$ , 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- $\kappa$ B-activation. In contrast, Tyr-305 phosphorylation by c-Abl has been implicated in I $\kappa$ B $\alpha$  nuclear translocation and inhibition of NF- $\kappa$ B-activation. Thus, tyrosine phosphorylation of I $\kappa$ B $\alpha$  may be an important regulatory mechanism in NF- $\kappa$ B signaling.

## References

- Bui, N.T. et al. (2001) *J Cell Biol* 152(4):753. (Background)  
 Waris et al. (2003) *J Biol Chem* 278(42):40778. (Background)  
 Naidu, S. et al. (2008) *J Immunol.* 181:4113. (WB: Mouse RAW264.7)  
 Sethi, G. et al. (2007) *Oncogene* 26(52):7324. (WB: H. epithelial H1299, Y42F)  
 Boosani, C.S. et al. (2007) *Blood* 110(4):1168. (WB: M. lung endothelial, MLEC)

## Immunogen:

I $\kappa$ B $\alpha$  (Tyr-42) synthetic peptide (coupled to KLH) corresponding to amino acid residues around tyrosine 42 of human I $\kappa$ B $\alpha$ . This peptide sequence has low homology to other I $\kappa$ B proteins, but does have some homology to unrelated proteins that may have a conserved tyrosine phosphorylation motif.

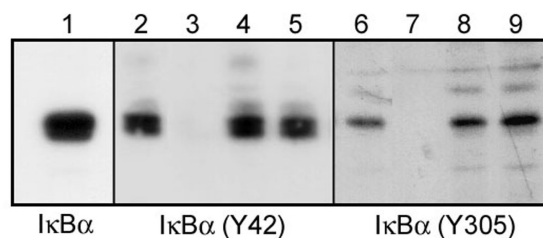
## Applications:

WB	1:1000
ELISA	1:2000
IP	1:100

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 1 hour at room temperature.

## Related Products:

IP1041	I $\kappa$ B $\alpha$ (Tyr-305), phospho-specific	Rabbit Polyclonal
IP1861	I $\kappa$ B $\alpha$ (C-terminus)	Rabbit Polyclonal
IX1035	phospho-I $\kappa$ B $\alpha$ (Tyr-42) Peptide	
IX1045	phospho-I $\kappa$ B $\alpha$ (Tyr-305) Peptide	
AL9401	A431 Pervanadate Ctrl Lysate	
AL9501	A431 + Pervanadate Lysate	



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

## Buffer and Storage:

Rabbit polyclonal, affinity-purified antibody is supplied in 100 $\mu$ l phosphate-buffered saline, 50% glycerol, 1 mg/ml BSA, and 0.05% sodium azide. Store at  $-20^{\circ}$ C. 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-42) peptide (without carrier). The antibody detects a 38 kDa\* protein on SDS-PAGE immunoblots of A431 and Jurkat cells treated with pervanadate, but not in control cells. Due to homologies to tyrosine sites on other proteins, it is recommended that the antibody be used to detect phosphorylation of immunoprecipitated I $\kappa$ B $\alpha$ .

\*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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Rev 1/2/2008