

# I $\kappa$ B $\alpha$ Phospho-Regulation Antibody Sampler Kit

Catalog # IK6320

## Kit Components:

| Catalog# | Description   | Host       | Size        | Applications | Species Reactivity | MW (kDa) |
|----------|---|------------|-------------|--------------|--------------------|----------|
| IP1031   | I $\kappa$ B $\alpha$ (Tyr-42), phospho-specific        | Rabbit pAb | 50 $\mu$ l  | WB, E, IP    | H, R, M            | 38       |
| IP1041   | I $\kappa$ B $\alpha$ (Tyr-305), phospho-specific       | Rabbit pAb | 50 $\mu$ l  | WB, E        | H, R, M            | 38       |
| IM3741   | I $\kappa$ B $\alpha$ (Ser-32/Ser-36), phospho-specific | Mouse mAb  | 50 $\mu$ l  | WB, E, IP    | H, R, M            | 38       |
| IP1861   | I $\kappa$ B $\alpha$ (C-terminus)                      | Rabbit pAb | 50 $\mu$ l  | WB, E        | H, R, M            | 38       |
| MS3001   | Anti-Mouse Ig:HRP                                       | Donkey pAb | 100 $\mu$ l | WB, E        |                    |          |
| RS3251   | Anti-Rabbit Ig Light-Chain Specific:HRP                 | Mouse mAb  | 100 $\mu$ l | WB, E        |                    |          |

Applications: WB = western blot, E = ELISA, ICC = Immunocytochemistry, IP = immunoprecipitation. Species: H = Human, R = Rat, M = Mouse

## Kit Summary:

The I $\kappa$ B $\alpha$  phospho-regulation antibody sampler kit can be used to detect I $\kappa$ B $\alpha$  phosphorylation on Ser-32/Ser-36, Tyr-42, and Tyr-305. The kit also includes a polyclonal antibody to monitor total I $\kappa$ B $\alpha$  expression levels and secondary reagents for detection of these antibodies.

## Buffers and Storage:

Rabbit polyclonal antibodies are supplied in 50 $\mu$ l phosphate-buffered saline, 50% glycerol, 1 mg/ml BSA, and 0.05% sodium azide. Secondary reagents are in the same buffer without azide. Mouse monoclonal (IM3741) is supplied in 50 $\mu$ l phosphate-buffered saline, 1 mg/ml BSA, and 0.05% sodium azide. Store all at -20°C. Stable for 1 year.

## Background:

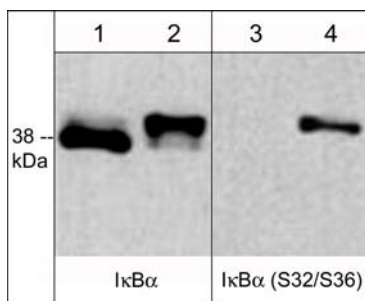
The NF- $\kappa$ B/Rel transcription factors are present in the cytosol in an inactive state complexed with the inhibitory I $\kappa$ B proteins. Activation of I $\kappa$ B $\alpha$  occurs through both serine and tyrosine phosphorylation events. Activation through phosphorylation at Ser-32 and Ser-36 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:

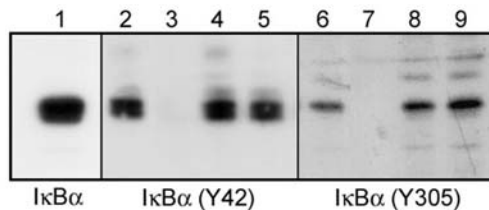
Bui, N.T. et al. (2001) J Cell Biol 152(4):753.  
Finco, T.S. et al. (1994) Proc. Natl. Acad. Sci. USA 91:11884.  
Waris et al. (2003) J Biol Chem 278(42):40778.

## Product References (IP1031):

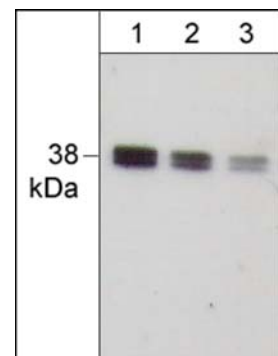
Naidu, S. et al. (2008) J Immunol. 181:4113. (WB: Mouse RAW264.7)  
Sethi, G. et al. (2007) Oncogene 26(52):7324. (WB: H. epithelial H1299, Y42F)  
Boosani, C.S. et al. (2007) Blood 110(4):1168. (WB: M. lung endothelial, MLEC)



Western blot analysis of Jurkat cells untreated (lanes 1 & 3) or treated with TNF $\alpha$  (1 nM). The blots were probed with anti-I $\kappa$ B $\alpha$  (lanes 1 & 2) or anti-I $\kappa$ B $\alpha$  (Ser-32/Ser-36) (lanes 3 & 4).



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.



Western blot of human A431 lysate. The blot was probed with anti-I $\kappa$ B $\alpha$  (C-term.) polyclonal antibody at a dilution of 1:500 (lane 1), 1:1000 (lane 2), or 1:2000 (lane 3).

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