

Phospho-IκBα (Ser32/36) (5A5) Mouse mAb

- Small 100 μl (10 western blots)
- Large 300 μl (30 western blots)

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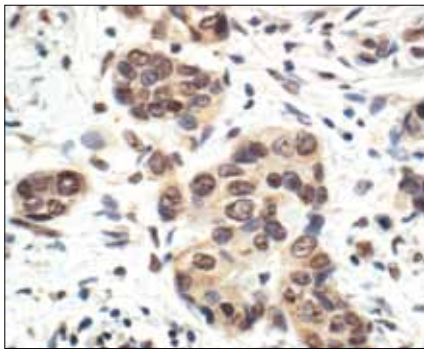
This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IHC-P Endogenous	H, M, R, Mk, (Dg, Pg, B, Guinea pig)	40 kDa	Mouse IgG1**

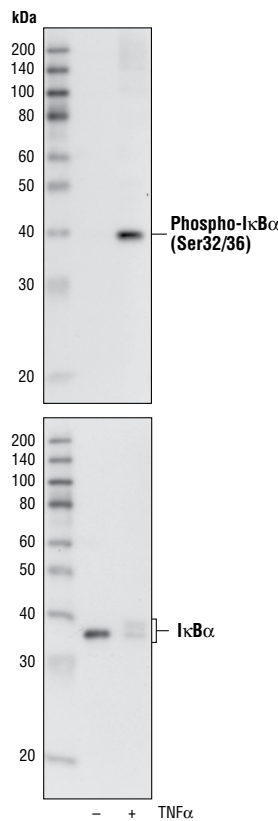
Background: The NF-κB/Rel transcription factors are present in the cytosol in an inactive state complexed with the inhibitory IκB proteins (1-3). Activation occurs via phosphorylation of IκBα at Ser32 and Ser36 followed by proteasome-mediated degradation that results in the release and nuclear translocation of active NF-κB (3-7). IκBα phosphorylation and resulting Rel-dependent transcription are activated by a highly diverse group of extracellular signals including inflammatory cytokines, growth factors and chemokines. Kinases that phosphorylate IκB at these activating sites have been identified (8).

Specificity/Sensitivity: Phospho-IκBα (Ser32/36) (5A5) Mouse mAb detects endogenous levels of IκBα only when phosphorylated at Ser32/36.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser32/36 of human IκBα.



Immunohistochemical analysis of paraffin-embedded human breast tumor using Phospho-IκBα (Ser32/36) (5A5) Mouse mAb.



Western blot analysis of extracts from NIH/3T3 cells, untreated or TNF-α-treated (#2169, 20 ng/ml) for 5 minutes, using Phospho-IκBα (Ser32/36) (5A5) Mouse mAb (upper) or IκBα (L35A5) Mouse mAb (Amino-terminal Antigen) #4814 (lower).

Entrez-Gene ID #4792
Swiss-Prot Acc. #P25963

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.

*Species cross-reactivity is determined by western blot.

**Anti-mouse secondary antibodies must be used to detect this antibody.

Recommended Antibody Dilutions:

Western blotting 1:1000
Immunoprecipitation 1:50
Immunohistochemistry (Paraffin) 1:1200†
Unmasking buffer: Citrate
Antibody diluent: TBST-5%NGS

Detection reagent: SignalStain® Boost (HRP, Mouse) #8125

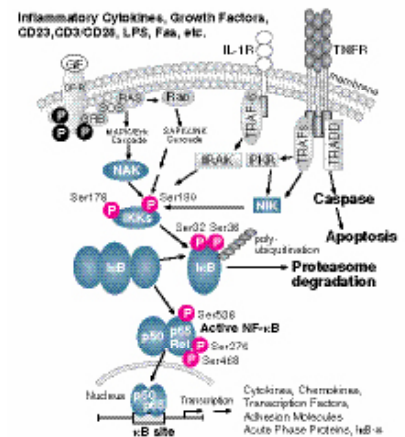
†Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.

For application specific protocols please see the web page for this product at www.cellsignal.com.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

- (1) Baeuerle, P.A. and Baltimore, D. (1988) *Science* 242, 540-546.
- (2) Beg, A.A. et al. (1993) *Genes Dev.* 7, 2064-2070.
- (3) Finco, T.S. et al. (1994) *Proc. Natl. Acad. Sci. USA* 91, 11884-11888.
- (4) Brown, K. et al. (1995) *Science* 267, 1485-1488.
- (5) Brockman, J.A. et al. (1995) *Mol. Cell. Biol.* 15, 2809-2818.
- (6) Traenckner, E.B. et al. (1995) *EMBO J.* 14, 2876-2883.
- (7) Chen, Z.J. et al. (1996) *Cell* 84, 853-862.
- (8) Karin, M. and Ben-Neriah, Y. (2000) *Annu. Rev. Immunol.* 18, 621-663.

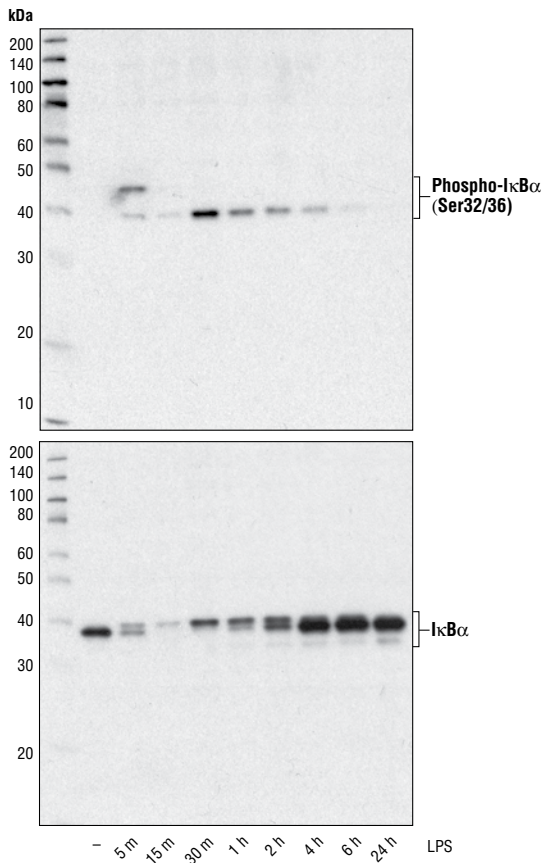


IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide

Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine

Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



Western blot analysis of extracts from THP-1 cells, differentiated with TPA (#9905, 80 nM for 24 h) and treated with 1 μ g/ml LPS for the indicated times, using Phospho-IκBα (Ser32/36) (5A5) Mouse mAb (upper) and IκBα (L35A5) Mouse mAb (Amino-terminal Antigen) #4814 (lower).